The Effects of Greek Yogurt and Exercise on Strength, Muscle Thickness and Body Composition in Untrained, University-Aged Males

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Abstract

Previous research has shown the effectiveness of milk/whey protein plus exercise on increasing muscle size, optimizing body composition and increasing strength in adult males and females. Greek yogurt (GY) contains similar muscle-supporting nutrients as milk yet it is different in several ways including being a solid food, and it has yet to be investigated in this context. Thus, the purpose of this study was to assess the effects of GY consumption plus exercise (resistance and plyometric) training on strength, muscle thickness and body composition. Thirty untrained, university-aged (18-25 years) males were randomized to 2 groups (fat-free, plain GY; n= 15, or a Placebo Pudding [PP; isoenergetic carbohydrate-based pudding]; n= 15) and underwent a combined resistance/plyometric training program 3d/week for 12 weeks. They consumed either GY (20 g protein per serving) or PP (0 g protein per serving) daily (GY: 3x200 g on training days and 2x150 g on non-training days; spread throughout the day). After 12 weeks, both groups significantly increased strength, muscle thickness and fat-free mass from baseline ($p<0.05$). GY gained more strength (GY; 26.8%, PP; 15.1%) than PP in 3 of 4 exercises determined by 1-RM ($p<0.05$). GY gained more biceps brachii muscular thickness (GY; 16.4%, PP; 7.1%) than PP determined by ultrasound ($p<0.05$). GY also increased fat-free mass (GY; 3.9%, PP; 2.3%) and reduced % body fat (GY; -1.1%, PP; 0.1%) more than PP determined by air-displacement plethysmography ($p<0.05$). Thus, consumption of GY during a training program resulted in improved strength, muscle thickness and body composition over a carbohydrate-based placebo. Given the benefits of consuming GY and its distinctiveness from milk, GY may offer a plausible, post-exercise, nutrient-rich alternative for positive strength, muscle and body composition adaptations.

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Abbreviations

%BF	% Body Fat
1-RM	One Repetition Maximum
AA	Amino Acids
CHO	Carbohydrates
CSA	Cross-Sectional Area
DXA	Dual Energy X-ray Absorptiometry
FFM	Fat-Free Mass
FM	Fat Mass
GY	Greek Yogurt
g/kg/d	Grams/ Kilogram (of body weight)/ day

MPB	Muscle Protein Breakdown
MPS	Muscle Protein Synthesis
PDCAAS	Protein Digestibility Corrected Amino Acid Score
PLY	Plyometric Training
PP	Placebo Pudding
RDA	Recommended Dietary Allowance
RT	Resistance Training

CHAPTER 1:

1.1 Introduction

The use of protein supplements to facilitate exercise adaptations has long been documented in human populations (1). Dairy protein, which is comprised mostly of casein and whey protein has been demonstrated as a favourable recovery and muscle building protein (2,3). This is in part due to the complete essential amino acid (AA) profile and adequate leucine levels which is primarily responsible for muscle protein synthesis (MPS) (4–6). Whey, which is rapidly absorbed and delivered to necessary tissues, such as exercised muscles, is able to provide AAs to the muscle to begin MPS soon after consumption (1,7). Casein, which constitutes 80% of dairy protein, is absorbed slower, and is able to provide the muscle with a sustained, positive influx of AA (8). The prolonged absorption is hypothesized to attenuate muscle protein breakdown (MPB), allowing for a net positive protein balance (or a less negative balance) over a prolonged period time (9). With these unique characteristics, dairy protein, in particularly milk, has been shown to be an effective beverage for facilitating favourable adaptations to resistance training (RT) (2,3). This poses the question; would other dairy products elicit similar adaptations to RT as milk?

Greek Yogurt (GY) has become a popular dairy product due to its high protein content (17 g/175 g serving in unflavoured/plain GY) which is created during the manufacturing and condensing process in which GY is made from regular yogurt (10). To date, no research exploring the combined effects of GY and exercise on strength, muscle, or body composition exists. One review identified only 2 randomized controlled trials (RCTs), both of which investigated the use of regular yogurt as a weight loss or weight management tool in overweight, female populations (10). One study found that regular yogurt consumption (3x 170 g serving/day, 5 g protein/serving) in women resulted in greater fat loss and lean mass retention after 12 weeks during energy restriction (and no exercise) versus a control group (11).The other study found that regular yogurt (4 g of protein/serving) in women was unable to further

decrease body fat greater than the placebo (isoenergetic sucrose beverage) after 16 weeks of an energy deficit with RT (12). Another study in young, normal weight, untrained females indicated no benefit of regular yogurt (with 5 g protein/serving, 3x/day) and RT on increasing strength or lean mass compared to a protein-matched control (13). The amount of protein provided by the regular yogurt was likely insufficient to produce results, particularly in studies with an exercise component. GY, however, contains 3-4x the amount of protein than regular yogurt, and thus warrants investigation in this context.

There is strong support for the use of isolated protein supplements, such as whey for increasing strength, muscle size, and lean mass while partaking in RT (1,7,14). However, research regarding whole-food protein sources is limited. It is important to study whole-food protein sources as they likely contain additional food components beneficial to overall health such as micronutrients, and the food matrix in whole foods may affect nutrient absorption (15). Hartman et al., (2007) and Josse et al., (2010) have shown that milk and RT was able to produce significant strength and body composition improvements compared to isoenergetic placebos (2,16). However, research by Rankin et al., (2004) found no benefit of chocolate milk on these outcomes compared to CHO (17). Similar to milk, GY contains important nutrients for musculoskeletal health such as calcium, phosphorus and protein, however the consistency and composition of GY is different from milk. GY possesses unique properties that may provide additional health benefits such as the provision of probiotics (18). Probiotic/fermented foods, such as GY, assist digestion, increase bioavailability of nutrients, and enhance immunity (10,18–22). In addition, in terms of the overall diet, by promoting GY consumption, other healthful eating behaviours may increase such as the consumption of more fruits, fibrous foods such as oats and granola, calcium, probiotics, and also improving eating behaviours by increasing satiety and normalizing appetite. All of these properties may potentially lead to increased health in addition to enhancing physical outcomes such as muscle strength, muscle thickness and body composition.

This thesis focuses on GY consumption with RT and plyometric (PLY) training on three main outcomes: muscular strength, muscle thickness, and body composition. A review of the current literature is provided, and due to the minimal research on GY or yogurt in general, the literature review also highlights research on milk and to a lesser extent whey protein.

1.2.1 Rationale for Investigating Greek Yogurt

The justification for this research was to explore a gap in the current understanding of the effects of a non-milk dairy food (i.e. GY) on variables relating to muscle strength, muscle thickness, and body composition following muscle/strength-building and plyometric exercise (RT and PLY) in young, untrained males. GY has recently become a popular dairy product due to its higher protein and decreased sugar content (plain GY) compared to regular yogurt (17 g vs 5 g of protein per 175 g serving, respectively). However, both types of yogurt are made from heating milk and introducing active cultures such as Lactobacillus bulgaricus and Streptococcus thermophiles to induce fermentation (23). Then, the regular yogurt undergoes a straining process which removes some whey and lactose thus concentrating the remaining contents (24). The final product is a thick, strained yogurt or what is commonly called GY. It provides more protein per gram (1 g protein per 10 g GY) making it a potentially favourable post-exercise whole-food source of protein. Currently, there is no research on the use of GY post-training on any measure relating to strength, muscle, and/or body composition. Thus, this thesis research investigated these outcomes with GY in a 12-week RT and PLY protocol in untrained, university-aged males. The study also included a placebo group that underwent the same training protocol but consumed a carbohydrate-based, isoenergetic pudding (placebo pudding: PP) which was designed to mimic the consistency of GY. This study design allowed us to indirectly compare to similar studies done using milk in the same population using a similar protocol (2,17).

Yogurt has many unique properties that make it somewhat different from milk and adds to the rationale for investigating it. Yogurt is available in a variety of flavours and lactose-free options and its

solidity makes it more satiating than liquid food sources (25). Yogurt can serve as a vehicle for consumption of other healthful foods such as cereals, nuts and fruits to form a complete meal (26). Since yogurt also has a longer shelf-life and stability compared to milk (27), it could serve as a more practical and dependable option for consuming dairy post-workout. Recent research has also linked fermented dairy products with a reduced risk for developing type 2 diabetes (28). Research has shown the positive effects of whey protein on muscular hypertrophy (1,29). However whey protein powder often lacks supporting nutrients which contribute to overall health such as calcium and magnesium, and probiotic cultures (23,30,31). Lastly, our research is applicable/translatable to the general population because we used yogurt that is available to consumers.

1.2 Rationale for Investigating Outcomes

The outcome variables of interest were selected because they are associated with improved health and disease prevention. Muscle strength describes the amount of force muscles can generate and is inversely associated with all-cause mortality in healthy men (32,33). By improving strength as a young adult, and maintaining it into late adulthood, an individual may decrease their risk of developing diseases or physical states associated with low muscular strength such as sarcopenia (34), osteoporosis (35), obesity (36) as well as physical and functional limitations (37,38) while maintaining their independence and autonomy (39,40). The accumulation of muscle tissue (or fat free mass (FFM)) is also associated with increased strength and athletic performance in addition to preventing or attenuating many chronic diseases such as type 2 diabetes and cardiovascular disease (41). This relates to the fact that muscle tissue plays a very important role in glucose homeostasis as it is the largest reservoir for glucose disposal and storage (42). Percent body fat, expressed as body fat mass relative to total mass, is a useful indicator of overall health (43). Negative associations exist between individuals who exercise more frequently and fat mass, with less fat mass in the upper and central body regions compared to infrequent exercisers (44). This reduces the risk for obesity and metabolic disorders (44). A body fat

percentage of 13-22% is considered normal for adult males (45). A high body fat percentage (>40th percentile) is associated with all-cause mortality risk (46).

CHAPTER 2: Literature Review

2.1 Population

The population chosen for this study was untrained, university-aged males. Since the investigation of GY and exercise is a new phenomenon, it was decided that it is best to start in a healthy, young sample to determine whether there is an effect on our variables of interest before investigating in a clinical population. A healthy population is, for the most part, void of any physiological abnormalities which could potentially deliver different findings. Untrained, which was defined as performing structured resistive exercises twice a week or less for at least the last 6 months, was chosen due to these subjects potentially being more sensitive to the training stimulus (47). This should maximize our chances of seeing training adaptations in the variables of interest following a 12-week training program. Untrained individuals are also more sensitive to training stimuli compared to athletes or conditioned individuals (48,49). A meta-analysis noted that strength gains can be made in untrained individuals with an intensity of 60% 1-RM and 4 sets per muscle group whereas athletic populations need to train at 85% 1-RM and 8 sets per muscle group (50). This meta-analysis noted the effort-to-benefit ratio for strength adaptations increases with training status. That is, as training experience advances, an individual needs to apply more effort to see comparable benefits as novice trainers (48,50). Since the current study was only 12 weeks in duration, we decided a novice population would be more sensitive to training adaptations than previously trained individuals. Young adults (18-25 years old) are also more sensitive to training stimuli, and have a lower threshold for MPS than elderly individuals who are more resistant to the anabolic effects of protein and thus require larger doses to elicit a similar MPS effect (5).

The reason we chose to only study males was to maintain a homogenous sample. From a physiological and hormonal standpoint, young adult males and females are different (51–53).

The magnitude and rate of hormonal changes in response to exercise differs between males and females (54,55), and therefore grouping males and females together would make interpretation of our results difficult. In young adults, interventions should be separated by sex (54), unless it is an outcome to compare between sexes. Future research could study this paradigm in a female population.

2.2 Greek Yogurt

Dairy products are nutrient dense and offer a favourable nutrient profile for optimizing strength and muscle while reducing body fat (56). The protein in GY is primarily casein (10,24), which allows for a prolonged influx of AAs which may theoretically assist in attenuating MPB (57). The acidity of yogurt delays gastric emptying, allowing prolonged time for nutrient absorption (58). Due to the lower pH of yogurt, dairy minerals such as calcium and magnesium are present in their ionic forms, which increases their absorption (59). Yogurt tends to be a more easily-digested and tolerable source of dairy compared to milk due to naturally occurring enzymes present in the bacterial cultures. These enzymes are responsible for the intra-intestinal digestion of lactose into its monosaccharide components glucose and galactose, making it more easily digested by lactose-intolerant populations (60). Yogurt consumption has also been shown to be positively associated with a reduced risk of developing type 2 diabetes. This may be related in part to its ability to delay gastric emptying, which subsequently reduces blood-glucose and insulin fluctuations (18,61).

The probiotic cultures added during yogurt production also offer health benefits. The bacterial cultures support healthy gut microflora and provide antipathogenic and anti-inflammatory properties (31). Yogurt has also been associated with beneficial immunological properties (62). Its consumption has been associated with a reduced risk of developing or experiencing symptoms of diarrheal-based diseases (63), colon cancer (64), irritable bowel syndrome (65) and food allergies (66). Bacteria within yogurt also contain proteolytic enzymes and peptidases which help to catabolize the proteins in yogurt making them more easily digested and absorbed (67). Dairy calcium has also been associated with

reducing FM (68). The combination of these nutrients and unique properties of GY make it an attractive and functional food for possibly improving important health parameters. However, research has yet to investigate the use of GY in this way and/or in combination with exercise. Due to the unique properties of GY, it is plausible that individuals beginning a RT program may yield similar strength, muscle and body composition adaptations from GY as other previously-studied protein sources, while also providing additional health benefits due to the bone-supporting and digestive nutrients present.

2.2.1 Protein Content

Milk, which consists of 80% casein and 20% whey protein (18), has been shown to result in a greater net protein balance and greater muscle protein accretion than an isonitrogenous and isoenergetic soy protein following one bout of RT (69). Chronic milk consumption in combination with a 12 week RT program increased strength and lean mass (fat and bone-free lean mass) while reducing FM compared to a CHO-based beverage in young male (2) and female (3) untrained individuals. However, there is conflicting research. Rankin et al. found no added benefit of young males consuming chocolate milk over a CHO placebo to increase lean mass or strength after 10 weeks of RT (17). Candow et al., found that there were no differences between whey and soy for increasing lean mass and strength following a 6 week RT protocol in young males and females (70). It is possible that these studies were too short to see divergent changes in the protein groups. As well, each group consumed 1.2 g/kg/d of protein throughout the study, leading the authors to conclude that protein source was not an important indicator of lean mass and strength. Similar research resulting in non-significant findings of dairy supplementation and RT featured total protein intakes below the recommended level for those in a RT program (≥1.6 g/kg) (71–73) and similar protein intake levels between the intervention groups which may explain why no interaction effect was present (13,17,74–77). Due to the relatively high protein content of GY in comparison to other protein-rich foods (Figure 2.1), GY may serve as a practical option to increase total protein intake and yield beneficial training adaptations.

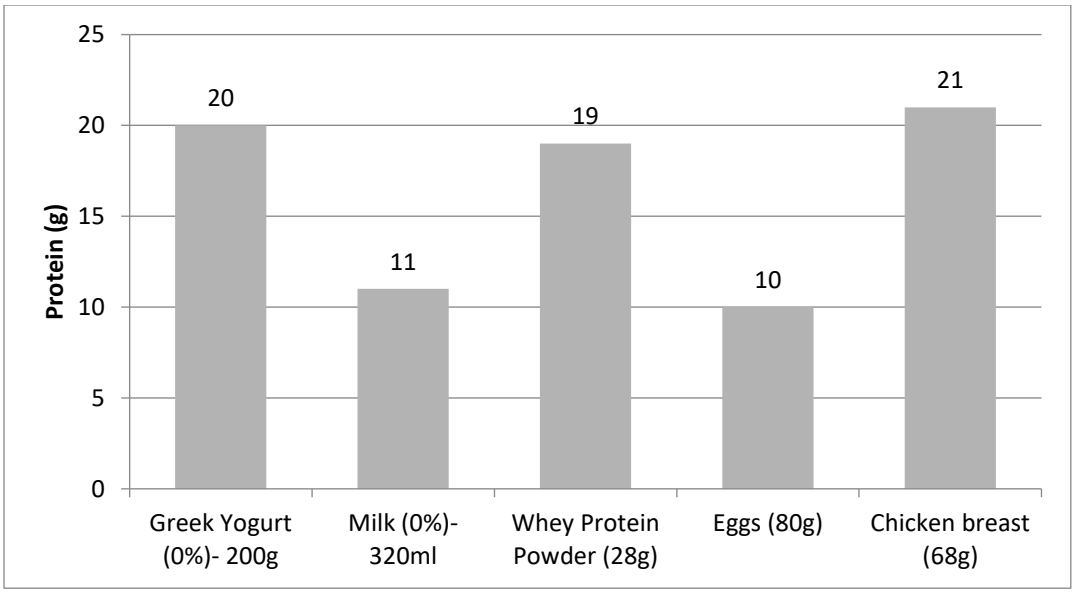

FIGURE 2.1- Popular energy-matched protein sources. Each food contains 113 kcals which was equivalent to one serving of Greek yogurt used in our study. Information obtained from ESHA (Food Processor, ESHA Inc., Salem, OR).

2.2.2 Dairy Protein Quality

In addition to total protein amount, protein quality is also important. High quality protein contains sufficient amounts of all essential AAs and is effectively digested and absorbed (78). Dairy and its constituent proteins can be scored based on their biological value (BV) which determines the efficiency of the protein to be synthesized in bodily tissues. Protein digestibility corrected amino acid score (PDCAAS) is another ranking system based on the completeness of AA within the protein and its digestibility (79). BV is out of 100, and is a relative score compared to egg protein. PDCAAS is out of 1.0. Bovine milk has a BV of 91, and PDCAAS of 1.00, Casein has a BV of 77 and a PDCAAS of 1.00, and whey has a BV of 104, and a PDCAAS of 1.00 (80). The Digestible Indispensable Amino Acid Score (DIAAS) is a relatively new protein quality scoring system that is currently accepted as more accurate for assessing protein quality (78). The DIAAS is based on the digestible content of the indispensable AA of a given protein (81). Research has indicated that the PDCAAS generally overestimates protein quality compared to the DIAAS (as assessed by true fecal nitrogen digestibility), especially in lower quality proteins (82). This new scoring system further demonstrates the high quality (scores >100 = high/excellent quality) of

dairy protein (milk protein concentrate= 120) over plant based sources such as soy and wheat (soy flour=89, wheat=45) (83). It is important to note that much of the research on protein supplementation and RT uses isolated protein sources, such as whey protein or soy protein. The combination of other macronutrients and micronutrients within a food may affect digestion and absorption (84,85). Thus, further research on GY is specifically required.

2.2.3 Protein Kinetics of Absorption

Casein protein exists in a micellar form which encases the casein molecule making it insoluble in water (57). This causes casein to coagulate in the stomach, resulting in prolonged digestion and ultimately slowing AA absorption into the blood (8). Whey protein is the acid-soluble portion of protein in dairy, and is rapidly digested and absorbed into the blood (8). The production of GY involves a straining process, which removes some of the whey protein. Although the exact whey to casein ratio of GY is not known, based on the production method, it is hypothesized that GY contains less than 20% whey protein and more than 80% casein. Fast-absorbing proteins like whey induce a rapid, but transient period of hyperaminoacidemia and leucine influx (86). Casein protein, which is absorbed slowly, induces a delayed, but prolonged AA influx. Postprandial leucine balance over 7 hours was higher after consumption of casein than a free AA mixture mimicking casein and a free AA mixture mimicking whey (87). This suggests that casein is able to promote a longer duration of positive net protein balance which would be favourable for MPS and MPB. This was confirmed in a different study using a stable isotope tracer method where the absorption kinetics of ^{13}C-leucine labelled whey and casein proteins were determined. The results of leucine's absorption kinetics from each protein support these findings (8). Specifically, protein breakdown was inhibited by 34% with casein ingestion, but not inhibited with whey. However, whey did increase whole-body protein by 68%, compared to 31% with casein (8). Figure 2.2 shows the leucine concentrations in the blood over time for both proteins. Casein was able to achieve a greater positive net leucine balance over 7 hours compared to whey (8). However, the rapid absorption of whey and

increased leucine content was able to cause a greater initial increase in MPS. Therefore, the effects of whey and casein proteins when consumed together, as is the case in dairy products, are complimentary, and provide both a rapid influx and subsequent absorption of AA into muscle fibers to support MPS soon after ingestion, as well as the prolonged influx of AA necessary to offset MPB and maintain a positive net protein balance hours later. Additional research supported these findings as whey and casein have been shown to produce equal overall MPS responses despite temporal differences in AA absorption (9).

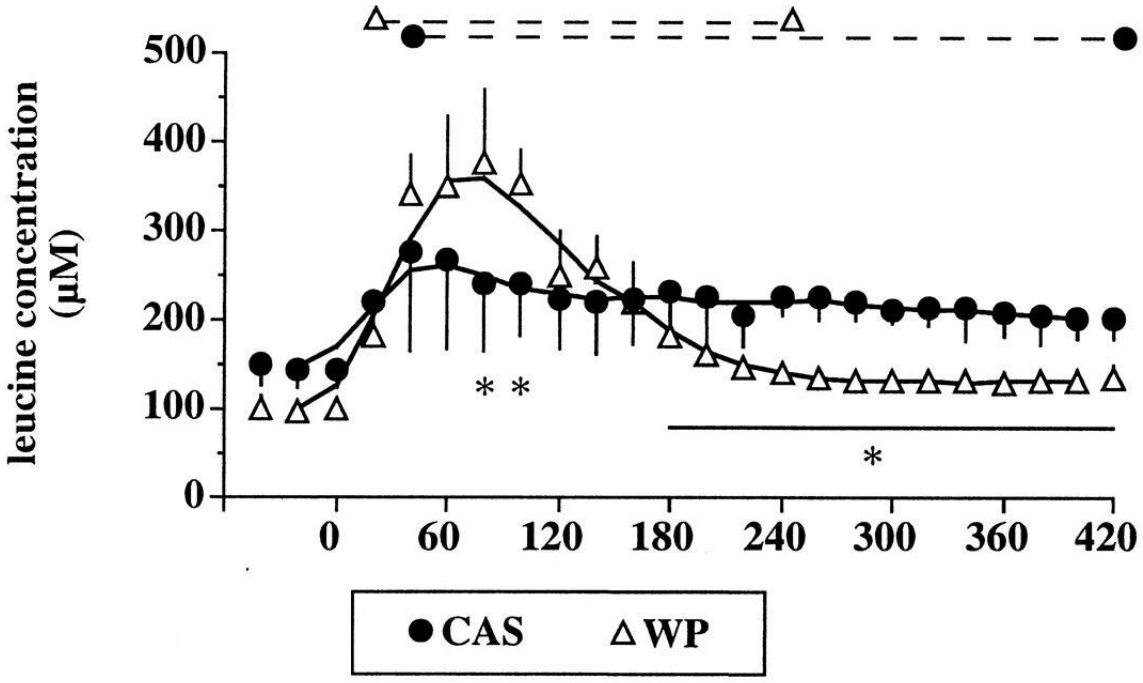

*FIGURE 2.2 - Protein Kinetics. This figure demonstrates the plasma-leucine concentration following whey and casein consumption over 7 hours. * denotes a significant difference between groups (<0.05). The dashed lines at the top of the figure denote a significant difference from baseline within group (<0.05)* (8).

2.2.4 Protein and Body Composition

In addition to building muscle tissue, protein has unique and advantageous properties which make it an attractive nutrient for improving and maintaining a healthy body composition. The majority of research regarding exercise and protein on body composition outcomes utilize protocols involving energy-restriction and are usually related to weight loss (88,89). RCTs by Hartman et al., (2007) and

Josse et al., (2010) demonstrated the positive effects of consuming milk with RT in promoting improvements in body composition with eucaloric diets in both males and females, respectively (2,3). The improvements in body composition with dairy were characterized by increases in lean mass paired with decreases in FM. A meta-analysis supports the use of whey protein in combination with RT as a means to improve body composition (7). A review on protein supplementation for weight loss and weight maintenance promotes intakes of 1.2 to 1.6 g/kg/day of protein with specific meals containing 25-30 g of protein (every 3-4 hours) for improving body composition (90). They arrived at this range by examining existing weight loss and weight management research that showed that individuals within this range for protein intake were more likely to lose weight and fat (and less lean mass) compared to those who consumed protein at the RDA which is 0.8g/kg/d (90). The review attributes these results to protein's increased thermic effect of food (which increases energy expenditure), and its effect on satiety and appetite suppression as being the main mechanisms by which protein can reduce fat mass. This, along with its ability to preserve lean body mass results in protein eliciting an overall favourable body composition change. Protein consumption has also been shown to preserve lean mass during short hypoenergetic periods (91). Of the three macronutrients, protein is more satiating than carbohydrates and fats (92) and requires more energy to metabolize (93). Research has demonstrated that high protein GY is able to reduce hunger, increase fullness and delay subsequent eating compared to other snacks containing lower levels of protein (18,94,95). Increasing satiety is a potential mechanism for optimizing body composition as it could limit overall food intake and thus decrease FM by promoting a negative (or less positive or neutral) energy balance.

In summary, the unique digestive properties of dairy proteins offer a rapid and prolonged influx of AAs essential for muscle growth (86). The satiating effect of protein as well as the dietary-induced thermogenesis appear to be beneficial for improving body composition. The ability of protein consumption alone to promote lean mass accretion can also stimulate an increased metabolism which

can improve body composition. Higher protein intakes (i.e. 40 g) seem to offer diminishing returns, as excess proteins are oxidized (96) and may contribute to increased fat mass *via* mechanisms involving gluconeogenesis and lipogenesis. This may ultimately cause undesirable changes in body composition (97).

2.2.5 Calcium and Body Composition

Higher calcium intake can increase lipolysis and decrease lipogenesis, resulting in fat loss (98). A meta-analysis in healthy males and females revealed a significant effect of dietary calcium on inhibiting fat absorption and increasing faecal fat loss (99). Additionally, research has shown that calcium derived from dairy foods is more effective than supplemental calcium at increasing fat loss possibly due to the additive effects of other bioactive compounds (68). Three or more servings of dairy/day has been associated with significant reductions in fat mass with energy restriction (100), and without energy restriction (68). Calcium (1400 mg/d) was also shown to significantly increase fat oxidation greater than 500 mg while on an energy deficit analyzed in a room calorimeter for 24 hours (101). Research providing obese subjects (low habitual calcium consumers ~400-500 mg/day) with three daily servings of 170 g of regular yogurt (providing 1100 mg/day) for 12 weeks resulted in significantly greater fat losses than an energy-void placebo (11). All subjects within this study were placed on a 500 kcal energy deficit diet and there was no exercise intervention. Research has also suggested that elevated calcium intake, along with higher protein intakes may further enhance fat loss due to their compounded effects (102). However, long-term studies are required to assess the effects of chronic calcium intake on fat and weight loss and to establish a dose-response relationship (99).

2.3 Protein Dose and Timing of Intake

The RDA for protein is 0.8 g/kg/d, however these values are based on the needs of sedentary individuals and reflect the amount of protein needed to replace typical losses. They do not consider the

increased requirements for individuals involved in RT and seeking to gain lean mass. 1.6 g/kg/day of protein is recommended to optimize training adaptations in untrained individuals (71,72). A review on protein doses found 20 g of high quality protein to be a sufficient amount for inducing hyperaminoacidemia which is able to augment MPS stimulated by RT (103). Another recent review by Schoenfeld et al., (2018) noted that the majority of existing research on protein dosage was obtained through analyzing fast-digesting sources of protein (such as whey) in the absence of other nutrients which may affect digestion and absorption rates (73). The author suggested that combining slower-digesting proteins and consuming them with other macronutrients may further delay absorption resulting in an enhanced utilization of AAs and a decrease in AA oxidation (73). This research demonstrated that in order to maximize anabolism, 0.4 g/kg should be the minimum amount of protein consumed per meal spread out across at least 4 meals per day to achieve the minimum threshold of 1.6 g/kg/day for individuals involved in RT (73). According to the minimum recommendation (73) for exercising individuals, an individual with body weight of 70 kg would need to consume 112 g of protein throughout the day, spread out over 4 meals containing 28 g of protein each. This could be partly achieved by providing participants with three 200 g servings of GY throughout the day which would provide 60 g protein over the day. These doses, in combination with one's habitual protein intake should allow individuals to meet these new daily protein intake recommendations and maximize adaptations.

Protein doses of 20 g in young adult male populations (which is present within 200 g of GY) are sufficient for stimulating MPS without increased oxidation and contributing to increased urea production (86,103–106). Moreover, research suggests that frequent, moderate whey protein consumption of 4 doses of 20 g, given every 3 hours following RT is optimal for MPS compared to two 40 g doses 6 hours apart or eight 10 g doses every 1.5 hours (107). In this study, although total protein was equalized, doses of 20 g every 3 hours appeared to maximize a positive net protein balance, optimizing MPS while also allowing for the consumption of sufficient amounts of total daily protein (1.6-1.7 g/kg/d)

(71,108). This level of consumption should also minimize excess protein oxidation, as research has indicated that a protein dose-dependent relationship may exist in untrained (106) and trained males (105). A recent study gave young adult males varied amount of protein (crystalline AA modelled after egg protein) with labelled [1-^{13}C]phenylalanine following intense exercise (shuttle run) to analyze whole body net protein turnover *via* breath and urine (109). Results indicated that net protein balance was achieved at approximately 0.15 g/kg/hour, suggesting that an anabolic limit exists whereby protein intake beyond this amount is oxidized. For a 70 kg individual, this equates to 10.5 g of protein per hour. However, this amount may be different following a full-body bout of RT and if AA absorption was delayed by a slower digesting protein such as casein.

Research providing 0, 10, 20 and 40 g of protein following RT in trained adult males demonstrated diminishing returns from consuming 40 g of protein as myofibrillar MPS did not differ from that of the 20 g dose. Instead, AA oxidation and urea production increased suggesting the excess protein was used elsewhere in the body (105). These findings are congruent with similar research which also examined the dose-response with protein intake following RT (see figure 2.3 below) (96). Similar methods using lean beef (30 g versus 90 g protein) supported previous findings in that no differences in MPS between conditions occurred at rest (104).

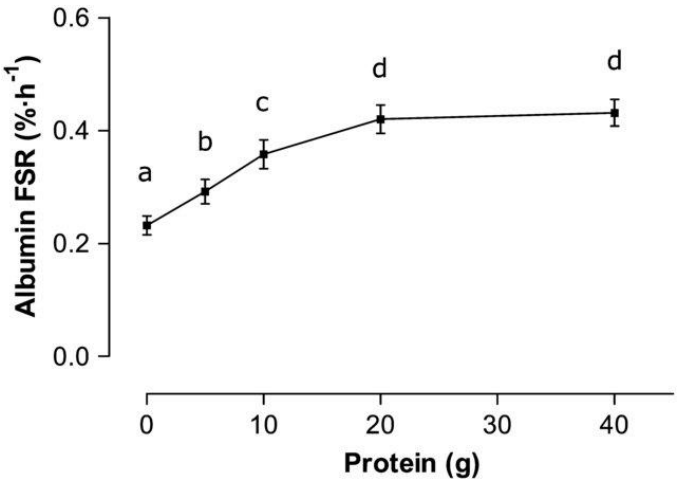

FIGURE 2.3 – Depicts the dose response relationship for protein (egg protein) following RT in young males. Means with different letters are significantly different from each other. (96)

The amount of total protein may not be as important as the leucine content within the protein. A review of protein quality and RT suggests that the leucine content of protein is important for maximally stimulating MPS and developing muscle mass during chronic RT (110). Leucine is known as the primary AA involved in MPS due to its ability to stimulate the mTOR signalling pathway (4,6,111). Research suggests 3 to 4 g of leucine is needed to maximally stimulate MPS in young populations (112,113). The Whey Protein Institute states that there is 1 g of leucine per 100 g of GY (114). Therefore, consumption of 600 g of GY (and 400 g within one hour post-exercise), would provide 4 g of leucine during this critical time. Supplementing with 4 g of leucine daily has been shown to increase strength following a 12 week RT program in untrained young men compared to a CHO placebo (115). However, an 8 week RT program in young untrained males supplemented with 3 g of leucine post exercise (2x/week) did not lead to greater strength or muscle size compared to the placebo (cornstarch) (116).

It is evident that RT acts as a primer for MPS by preparing the muscle for aminoacidemia (117). RT increases AA transporters to the cell membrane and increases blood flow to the working muscle (118). The sensitivity following RT for MPS, also known as the 'anabolic window', is thought to last for at least 24 hours, with no difference between trained or untrained individuals (105). Earlier research suggested that protein ingestion soon-after exercise could significantly enhance MPS greater than

protein ingestion during other time points (119). Research on untrained, young adult males participating in a 21-week RT program showed 15 g of whey protein consumption before and after training resulted in increased cell-cycle kinase cdk2 mRNA expression within the muscle (120). This is a marker of cell proliferation necessary for muscular hypertrophy, and suggests that protein consumption timed around RT resulted in higher cell proliferation within the muscle compared to a non-energetic placebo (water with colouring and sweetener) (120). Andersen et al., (2005) showed that a pre/post RT protein dose was able to significantly increase muscle size and strength compared to a carbohydrate beverage after 14 weeks of training in untrained males (121). Willoughby et al., (2007) also demonstrated that pre and post RT doses (40 g protein total) were able to increase muscle size, strength and body composition greater than the placebo (carbohydrate) after 10 weeks of RT in young, untrained males. In both the Andersen and Willoughby studies, it is possible the results were due to the protein group consuming more total protein than the carbohydrate group and thus the total amount of protein could be responsible for the increased muscle size and strength reducing the importance of timing.

Hoffman et al., (2009) designed a study to analyze the difference between pre/post RT protein consumption and morning/night protein consumption (122). Thirty-three resistance-trained young males underwent RT 4 days per week for 10 weeks. One group consumed protein before/after exercise, another group consumed protein at bedtime and in the morning and a third group only exercised with no protein. Each protein dose consisted of 42 g of protein. Food diaries and nitrogen balance *via* urinary analysis was implemented to measure total protein intake. The results indicated that timing of protein intake had no effect on strength, power, or body composition as all groups improved from baseline but did not significantly differ from each other (122). Both supplement groups were consuming protein beyond 2 g/kg/d and even the control group had a habitual protein intake of more than 1.5 g/kg/d which may be the reason why no differences were seen in the variables of interest. Another recent study showed no difference of 35 g casein supplementation at daytime versus nighttime, as both groups

were significantly able to increase muscle size, lean mass, and strength with no differences between groups (123). Both groups within this study were consuming 1.8 g/kg/d of protein daily leading the authors to suggest that total protein amount, if you are consuming above the recommendation, is likely more important than timing.

Current research also supports the use of pre-sleep protein ingestion during RT to further augment muscle size and strength gains by increasing overnight MPS and protecting against MPB (124). The sleeping hours are usually characterized by an overnight fast of around 6-10 hours, and during this time, MPS decreases and MPB increases. However, if protein is consumed right before bed (particularly protein that is slowly digested and absorbed), this may increase AA delivery to muscles during the night (57). As mentioned previously, GY is primarily comprised of casein protein which allows for a sustained influx of AA over time which has been shown to increase MPS while inhibiting MPB (8). Research by Snijders et al. (2015) assessed the effects of pre-sleep protein consumption and RT on strength and muscle size. The study found that following 12 weeks of RT in young, untrained adult males, the group consuming casein protein (27.5 g) prior to sleep experienced greater strength and muscle size gains compared to the noncaloric placebo (water) group (125). However, the daily protein intakes by the groups were not otherwise matched (protein group consumed 1.9 g/kg/d while control group consumed 1.3 g/kg/d), therefore these results cannot be attributed only to a pre-sleep protein dose. Despite this, an earlier, acute study from this lab demonstrated that casein protein prior to sleep was able to promote an increased blood-AA concentration and attenuate MPB throughout sleep (57).

A meta-analysis on 43 studies and 1003 participants revealed a small to moderate effect of protein timing (within 1 hour post-training) on strength and muscle hypertrophy (126). However, as shown in Figure 2.4, when controlling for covariates such as total protein intake, the significant relationship between timing and muscle hypertrophy dissipates. The meta-analysis indicated that protein timing is less important for increasing strength and muscular hypertrophy when consuming an

adequate amount of total protein while participating in a RT program (126). That is, a steady intake of protein throughout the day amounting to a total intake greater than 1.6 g/kg/d may be the most important factor in augmenting training adaptations.

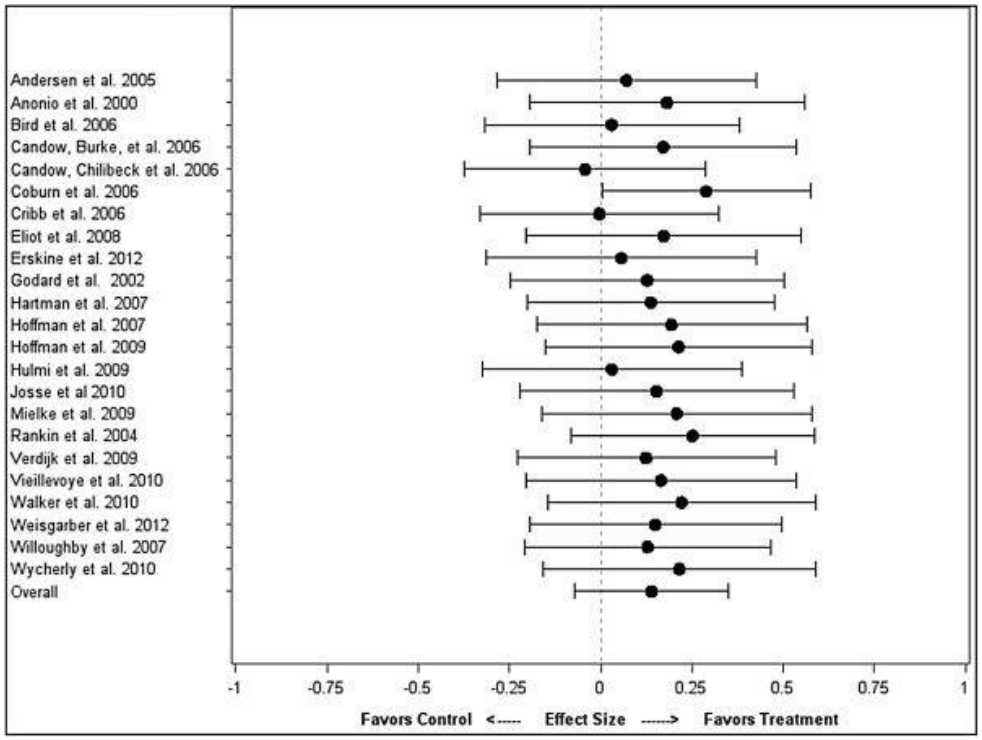

FIGURE 2.4 - The impact of protein timing on muscular hypertrophy when adjusted for total protein intake. There is a lack of data supporting the hypothesis for immediate protein consumption following RT and existing data support the theory that total protein intake is more important for increasing strength and muscle hypertrophy (126).

Energy provided following exercise alone has been shown to attenuate MPB and sustain a positive net protein balance (127,128). Comparing the effect of protein post-exercise to a carbohydrate-based placebo has been done before in other training studies (2,3,121,129–132,13,17,29,70,74–77). This allows for the direct detection of the effect of protein or the protein-containing food on outcomes of interest rather than being confounded by the lack of provision of calories to a placebo group. Research by Roy et al., investigated the acute effects of glucose consumption immediately and 1 hour following a unilateral knee extension exercise bout in young men. The study showed that carbohydrate

consumption following exercise was able to attenuate myofibrillar breakdown and urinary urea excretion which suggests a more positive net protein balance compared to the noncaloric placebo (133).

In summary, although timing the intake of protein around exercise may still be beneficial, research indicates that total protein intake of around 1.6 g/kg/day for novice exercisers may be the most important factor to facilitate strength and muscle adaptations in response to RT (72,108,126). Additionally, protein intakes of approximately 20-25 g or 0.25-0.4 g/kg/day 3-4 times per day (i.e. as meals) may be optimal to maximize MPS while limiting protein oxidation (71,72,108,134). The amount of leucine present in protein is also important to consider as this AA plays a significant role in MPS (5,113,135). Protein timing appears to have a small to moderate effect on increasing muscle hypertrophy and strength when consumed around the RT bout (126). Therefore, the primary focus should be placed on consuming adequate total protein, with considering timing of protein intake around RT secondary.

2.4 Training Prescription

RT is a form of training which utilizes resistance against muscular contractions with the goal of increasing strength, anaerobic fitness and skeletal muscle size. There are several factors able to mediate muscle size/growth including mechanical tension, muscle damage and metabolic stress (136). Each of these factors can be targeted through RT. Muscle tissue's hypertrophic response to RT is highly dependent on various training variables such as intensity, volume, exercise selection, and rest intervals (136). Muscle size increases in response to the RT stimulus. This is achieved by stressing the myofibers which leads to adaptations including the proliferation of contractile proteins such as actin and myosin and increases in the extracellular matrix to support muscular hypertrophy (137,138). Along with structural enhancements, RT also signals neurological adaptations, which are also able to increase muscular strength (72).

RT also has beneficial properties capable of enhancing body composition. RT has been shown to increase total energy expenditure by increasing resting metabolic rate as well as energy costs associated with training (139). RT also promotes maintenance or growth of metabolically active tissue, such as muscle, which may help to decrease fat mass and subsequently improve body composition (140–142). Circuit RT which consists of completing consecutive sets of different exercises with minimal rest in between, has demonstrated decreases in fat mass, and body fat %, while increasing fat-free mass in moderately active young adult males following a 12 week training program compared to non-training controls (143). A study in college-aged males demonstrated that RT was able to increase lean mass, strength and limb circumference significantly greater than endurance training and no training after 24 weeks (144).

Plyometrics (PLY), another form of exercise training, consists of explosive jumping exercises with the goal of muscle contraction from an elongated state to its shortened state as quickly as possible. A meta-analysis on PLY training showed this modality to increase power; a specific sport-performance factor (as measured by vertical jump height), which is a combination of speed and strength (145). Research in young, untrained men who underwent 12 weeks of either RT or PLY both experienced significant muscle size gains and strength gains, whereas power only increased within the PLY group (146). Similar research in young men that included a RT, PLY and RT+PLY group found that strength and power significantly improved to a greater extent in the combined training group than in each of the exclusive training groups following 12 weeks of training (3x/week) (147). PLY and RT were both included in the training prescription in this thesis study. This style of mixed-mode training may better reflect the nature of physical activity or sport in the general population (148). Additional details regarding the exercise training program are in Appendix 8.1.

2.5 Summary of the Literature

Below is a summary of current research on protein supplementation versus a placebo during a chronic RT program (minimum 6 weeks) on strength, muscle thickness, and/or body composition in young, untrained adults of normal body weight. Generally, results demonstrate that protein supplementation following RT is able to increase strength (3,70,121,125,129,130,132), muscle thickness (2,76,121,129) and muscle mass (2,3,29) greater than a placebo (which is usually an energy-matched carbohydrate beverage or water). Of the few studies that did not show significant interaction effects, these studies may have been too short in duration (< 10 weeks) (13,17,74,75,149), or total protein intake may have been insufficient (<1.6 g/kg/d) or not greater/different from the placebo group (13,17,75,77).

Table 2.1: Summary of Relevant Research

Study	Population and duration (RT sessions/ wk)	Protein Source	Protein Dose	Muscle Thickness	Fat-Free Mass	Muscle Strength
Hartman et al., 2007 (2)	56 Untrained males 12 wks; 5d/wk	Milk vs Soy vs Pla (CHO)	17.5 g 2x/day	↑*	↑*	↑
Snijders et al., 2015 (125)	44 Untrained males 12 wks; 3d/wk	Casein vs Pla (water)	27.5 g 1x/day	↑*	↑*	↑*
Andersen et al., 2005 (121)	22 Untrained males 14 wks; 3d/wk	Protein (whey, casein, egg) vs Pla (CHO)	25 g 1x/day	↑*	N/A	↑* jump performance only
Hulmi et al., 2009 (129)	31 Untrained males 21 wks; 2d/wk	Whey vs Pla (CHO) vs Con	15 g 2x/day	↑*	N/A	↑*

Willoughby et al., 2007 (130)	19 Untrained males 10 wks; 4d/wk	Whey+Casein vs Pla (CHO)	20 g 2x/day	N/A	↑*	↑*
Spillane et al., 2012 (149)	19 Untrained males 8wks; 4d/wk	BCAA vs Pla (CHO)	9 g 1x/day	N/A	↔	↑
Volek et al., 2013 (29)	63 Untrained males and females 32wks; 3d/wk	Whey vs Soy vs Pla (CHO)	22 g 1x/day	N/A	↑*	N/A
Candow et al., 2006 (70)	27 Untrained males and females 6wks; 4/wk	Whey vs Soy vs Pla	1.2 g/kg/day	N/A	↑* No difference in pro source	↑* No difference in pro source
Josse et al., 2010 (3)	20 Untrained females 12wks; 5d/wk	Milk vs Pla (CHO)	17.5 g 2x/day	N/A	↑*	↑*
Rankin et al., 2004 (17)	13 Untrained males 10wks; 3d/wk	Chocolate milk vs CHO	0.21 g/kg/day	N/A	↑	↑
White et al., 2009 (13)	35 Untrained females 8 wks; 3d/wk	Yog vs pro vs pla	5 g 3x/day	N/A	↑	↑
Vieillevoye et al., 2010 (76)	29 Untrained males 12 wks; 2d/wk	EAA + CHO vs Pla (CHO)	15 g EEA 2x/day	↑*	↑	↑
Bird et al., 2006 (131)	32 Untrained males 12 wks; 2d/wk	EAA + CHO vs Pla (CHO)	6 g EAA 1x/day	N/A	↑*	↑
Coburn et al., 2006 (132)	33 Untrained males 8 wks; 3d/wk Knee extension only	Whey + Leu vs Pla (CHO)	20 g whey + 6.2 g leu x1/day	↑	↔	↑*
Erskine et al., 2012 (77)	33 Untrained males 12 wks; 3d/wk Elbow flexion only	Whey vs Pla (CHO)	20 g x2/day	↑	N/A	↑
Weisgarber et al., 2012 (74)	17 Untrained males and females 8 wks; 4d/wk	Whey vs Pla (CHO)	0.3 g/kg/day	↑	↑	↑

*↑ indicates a significant positive improvement over time; * Indicates significant difference from placebo (Pla) group (interaction); N/A indicates variable not reported/measured.*

2.6 Rational for Study Design

The current study was designed according to previous research. Regarding protein, we gave participants GY which provided 20 g of protein per dose as this amount has been shown to maximally stimulate MPS while attenuating excess AA oxidation (96,105,150). Although the literature states a weak to moderate effect for consuming protein immediately post-exercise (126), we chose to give participants protein during this time to partly control supplement consumption adherence and to mimic the design of similar studies using milk for comparison purposes (2,3). Due to the specific digestion and absorption kinetics of casein protein (which comprises the majority of protein in GY), we gave participants GY (200 g on training days, 150 g on non-training days) prior to sleep as research shows that protein, specifically casein, prior to sleep may attenuate MPB and maintain an elevated net protein balance throughout sleep (57), and chronically lead to increases in muscle mass, muscle size and strength compared to a placebo (125). In addition to protein timing, we also wanted to ensure that our supplementation of GY allowed participants to meet the overall protein intake of 1.6 g/kg/day in efforts to maximize training adaptations (71–73,108).

Regarding our training protocol, we opted to have participants complete two full body RT sessions per week as research indicates this is a superior frequency as opposed to once per week for increasing muscle hypertrophy and strength (134,151). Within each RT session, each major muscle group was targeted through two exercises, each with 3-4 sets (152) of 8-10 repetitions at ~70% 1-RM. Most of these sets were taken to voluntary failure as research indicates that taking sets to voluntary failure induces greater hypertrophic responses than the completion of a predetermined number of reps at higher loads (153–156). The training was periodized and training variables were manipulated throughout the intervention to ensure continuous progressions were made (157–159). We included PLY training in addition to RT primarily because of its effects on bone health (which was a separate outcome

measure of this study and not related to this thesis) (160). The combination of RT + PLY may also be indicative of typical physical activity patterns in our specific population (148).

Lastly, regarding duration, we chose 12 weeks for our training intervention as this length allows for significant results to manifest (if present) while also considering subject compliance. Studies involving protein supplementation of 9 weeks or greater have been shown to be more effective than those <8weeks at inducing strength and muscle mass changes following RT with a nutritional manipulation (161).

CHAPTER 3: OBJECTIVE AND HYPOTHESES

3.1 Objective

To compare the effects of Greek yogurt to an isoenergetic, protein-void placebo pudding in combination with 12 weeks of resistance and plyometric training on strength, muscle thickness, and body composition in untrained, university-aged males.

3.2 Hypotheses

Although we anticipated that both groups would experience favourable exercise training adaptations, we hypothesized that 12 weeks of GY consumption with resistance and plyometric training would facilitate significantly greater increases in strength, muscle thickness, and fat-free mass while reducing fat mass compared to the consumption of an isoenergetic, protein-void placebo pudding.

CHAPTER 4: Methods

4.1 Participants

Healthy, university-aged (18-25y) males (n=30) were recruited for the study. Participants were untrained, defined as not being consistently involved in a RT program within the last 6 months where they were undergoing training more than twice per week. Participants were <25% fat, and were not currently taking any protein, vitamin and/or mineral supplements.

4.2 Experimental Design

This study was a parallel randomized controlled trial (clinical trial registration #: NCT03196856). Subjects were randomly assigned to one of two groups; Greek yogurt (GY; n=15) or placebo pudding (PP; n=15). Participants randomized to the GY group consumed 200 g of Oikos 0% Plain GY (~110 Kcals, 20 g protein, 8 g CHO) 3 times per day on training days (immediately and 1 hour post exercise and before bed) and 150 g, 2 times per day on non-training days (breakfast and before bed). The non-training day dose (300 g yogurt) was decided on to reflect the current dairy recommendation of 2 servings per day for the age and sex of the participants (162). In fact, 300 g of yogurt corresponds to just under 2 servings of dairy (which would be 350 g). Participants had the option to flavour the GY with calorie-free sweeteners/syrups if they preferred. The placebo group consumed a PP, which was an isoenergetic, chocolate flavoured, carbohydrate-based semi-solid food (~110 Kcals, 0 g protein, 28 g CHO) on the same schedule as the GY group. Nutrition facts for GY and PP can be seen in Figure 4.1 below. The PP was comprised of maltodextrin (2 parts), chocolate pudding powder (1 part), and water, and was designed to resemble the consistency and texture of GY. The PP was always made by the same person (A. Bridge). To ensure anonymity of the placebo, it was termed the '*study-designed supplement*' and its contents were kept discreet to participants and trainers. Both groups had their respective supplements divided into individual serving containers and labelled by study personnel. The post-exercise doses were

consumed in the laboratory following training with study personnel present, whereas the non-training day and before bed doses were consumed away from the laboratory and/or at home. These supplements were given to the participants to take home on a weekly basis. During the study, both groups were encouraged to maintain their habitual diets, with the exception of the intervention food. Participants were provided with the same information/advice to help them compensate for the added calories consumed from the supplements.

OIKOS 0% PLAIN GREEK YOGURT

Nutrition Facts

Serving size	(200g)
Amount Per Serving	
Calories	**110**
	% Daily Value*
Total Fat 0g	**0%**
Saturated Fat 0g	**0%**
Trans Fat 0g	
Cholesterol 10mg	**4%**
Sodium 70mg	**3%**
Total Carbohydrate 9g	**3%**
Dietary Fiber 0g	**0%**
Total Sugars 5g	
Includes 0g Added Sugars	**0%**
Protein 19g	**39%**
Vitamin D 0mcg	0%
Calcium 229mg	20%
Iron 0mg	0%
Potassium 0mg	0%

*The % Daily Value (DV) tells you how much a nutrient in a serving of food contributes to a daily diet. 2,000 calories a day is used for general nutrition advice.

PLACEBO PUDDING

Nutrition Facts

Serving size	(47g)
Amount Per Serving	
Calories	**110**
	% Daily Value*
Total Fat 0g	**0%**
Saturated Fat 0g	**0%**
Trans Fat 0g	
Cholesterol 0mg	**0%**
Sodium 0mg	**0%**
Total Carbohydrate 29g	**10%**
Dietary Fiber 0g	**0%**
Total Sugars 29g	
Includes 0g Added Sugars	**0%**
Protein 0g	**0%**
Vitamin D 0mcg	0%
Calcium 0mg	0%
Iron 0mg	0%
Potassium 0mg	0%

*The % Daily Value (DV) tells you how much a nutrient in a serving of food contributes to a daily diet. 2,000 calories a day is used for general nutrition advice.

FIGURE 4.1*—Nutrition facts tables for the respective study supplements.*

Both intervention groups underwent 12 weeks of exercise training, 3 times per week, in the campus gym (the Zone) or in equipped research laboratories (WH144, WH16) at Brock University. All training was facilitated by competent and certified trainers and/or senior kinesiology students guided by A. Bridge. Each formal training session (~60 min) consisted of either full-body RT (twice per week) which included free-weight and machine-based exercises including leg press, bench press and seated row (at ~70% 1-RM, 8-10 total exercises), or PLY training (once per week) which included exercises such as box jumps and frog jumps which were adjusted based on individual abilities and progressed accordingly (150-250 total jumps/impacts per training session). Each session consisted of a warm-up (5 min),

exercise (50 min), and a cool down with stretching (5 min). The training followed an undulating periodization protocol and utilized the principles of progressive overload, varying intensity and/or volume throughout the intervention. RT exercises were taken to voluntary failure (or close). The goal was to have participants succumb to failure between 8-12 repetitions in an effort to standardize training volume.

4.3 Measures

4.3.1 Questionnaires

After signing an informed consent document, participants filled out three different questionnaires. 1. A participant Screening and Medical History Questionnaire was completed by every participant before completing any other measures (Appendix 8.2). This questionnaire allowed us to have a better understanding of the participant's history to assess whether they were able to participate in the study and undergo all the required measurements. 2. The Godin Shephard Leisure Time Physical Activity Questionnaire which assessed habitual leisure-time physical activity levels (Appendix 8.3). This allowed us to gauge the participant's pre-study physical activity levels. 3. A PAR-Q (physical activity readiness questionnaire) which determined readiness to begin an exercise program was completed before the intervention (Appendix 8.4). All questionnaires were explained, administered, and analyzed by the same researcher.

4.3.2 Food Diaries

Participants recorded their baseline habitual food and drink intake for 7 days (consecutively) prior to beginning the intervention (Appendix 8.5). Participants completed a 3-day food diary during week 12 of the intervention. The 3-day food diary consisted of 2 weekdays and 1 weekend day. Instructions on how to fill out a food diary which discussed portion sizes, common measurements and strategies for providing a detailed description of foods eaten were provided to each participant in

advance by the same researcher. Dietary intake was inputted and analyzed using a diet analysis program (Food Processor, ESHA Inc., Salem, OR) by the same researcher.

4.3.3 Muscular Strength

Muscular strength was evaluated *via* voluntary 1 repetition maximum (1-RM) testing of four exercises at baseline and following week 12 of the intervention. 1-RM total was also calculated based on summing the 1RMs from the four exercises together. 1-RM testing is a validated assessment of muscular strength (163), and knowing an individual's 1-RM or predicted 1-RM is important when designing training protocols and monitoring progression (164). On both occasions, participants were instructed to come to the laboratory well hydrated and fed, and to not participate in any structured exercise for a minimum of 48 hours prior to testing. 1-RMs were determined for the following exercises: chest press, seated row, leg extension, and hamstring curl. Participants were made familiar with the exercises and testing protocol by doing light (estimated 40-50% 1-RM), practice repetitions before the actual pre- and post-intervention assessments began. During the assessment, weight was progressively added to each exercise until 1-RM was determined. Rests of 2-3 minutes were given between each attempt. Failure was determined when participants were unable to complete the full range of a repetition without compensation. If 1-RM was not determined after 4 consecutive attempts, it was estimated using the O'Connor calculation (1-RM = weight x (1+ (0.025 x reps))) (165,166) from the set with the lowest number of completed reps. The use of a predictive equation for estimating 1-RM has been previously validated in a young, untrained male population (166,167). When predicting the 1-RM, no more than 10 repetitions should be used, as research shows the predictive accuracy decreases after 10 repetitions (168). Weight was adjusted so that most participants experienced voluntary failure between 1 and 4 repetitions. All 1-RM assessments were completed by the same researcher.

4.3.4 Muscle Thickness

Muscle thickness was measured *via* ultrasonography (General Electric Medical Systems, Ultrasound Vivid I portable, Milwaukee, WI, USA) as the direct diameter through the centre of the target muscle at a specific anatomical location. Muscle thickness was measured at 2 locations: the rectus femoris + vastus intermedius (quadriceps) and the biceps brachii. Muscle thickness for the quadriceps was measured at 50% between the greater trochanter and lateral epicondyle of the femur. For the biceps, muscle thickness was measured at 40% from the proximal end between the greater tubercle and the lateral epicondyle of the humerus. These sites correspond to where the muscle belly is the thickest. These landmarks were marked on the participant, so the ultrasound probe could be placed directly on the marked site. A thin layer of gel was applied to each muscle site. The ultrasound probe was placed perpendicular to the skin on the site. The measurement was obtained by pressing the probe gently on skin and moving it over the muscle. Muscle thickness was measured from the bone to the outer/superficial sarcolemma. Subjects laid in a supine position, relaxed, with palms facing into their body. Biceps measurements were taken first, followed by quadriceps. To ensure accuracy of measurements, 3 images were obtained for each site and then averaged to obtain a final value. Ultrasound tests were completed 48-72 hours following exercise and were taken in the morning with the participant fasted for a minimum of 10 hours to ensure exercise and/or variable food intake did not affect results. All muscle thickness measurements were taken by the same researcher.

4.3.5 Body Composition

Body composition was measured using air-displacement plethysmography *via* the Bod pod. (COSMED USA Inc., BODPOD, Chicago, Il.) The bod pod is an “egg” shaped unit that the participant sits in. The testing procedure begins with calibration of the empty chamber with a known volume. The participant, dressed in compression shorts and swim cap (the same outfit for each participant was used for the pre- and post-intervention measures), sits inside the unit for 45 seconds where their raw body

volume is determined as the volume of air displaced (the difference between the volume of the empty vessel and the volume of the vessel with the participant inside). All Bod Pod measurements were completed 48-72 hours following exercise and were taken in the morning with the participant fasted for a minimum of 10 hours to ensure exercise and/or variable food intake did not affect results. Determined body volume is entered into a pre-set algorithm accounting for body weight (participants are weighed on a scale before they enter the bod pod), height (measured on a stadiometer before they enter the bod pod), age, and ethnicity. The participant's body composition (fat mass, percent body fat and fat-free body mass) is then estimated. Research suggests that air displacement plethysmography is a reliable and valid assessment tool to evaluate body composition quickly and safely (169–171). All body composition measures were taken by the same researcher.

4.4 Statistics

To ensure our study was adequately powered, the following power calculation was used:

$$n = 2 \times \frac{\sigma^2 \left(Z\beta + \frac{Z\alpha}{2}\right)^2}{difference^2}$$

Table 4.1: *Power calculation results. Sample sizes needed to achieve adequate power for specific variables of interest.*

Reference	**Variable**	***Required sample size per group (n)***
Hartman et al., 2007 (2)	FBLM (fat/bone free lean mass)	25
	Leg extension strength	3
	Hamstring curl strength	5
Snijders et al., 2015 (125)	Quadricep muscle CSA	7
	Chest press strength	23
	Leg extension strength	16

Power calculations were carried out for all 3 primary outcomes (Table 4.1) using data from previous, similar studies (2,125). Results were averaged to yield a sample size of 13 participants per group. Data were analysed using SPSS (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Data were checked, and normality was confirmed by assessing measures of central tendency and homogeneity of variances, and sphericity. Data points that were more than +/-2 SD from the mean were categorized as outliers and removed. Missing data points (1 GY participant for all post-strength measures, 1 GY and 1 PP participant for post-ultrasound measures) were replaced with the series mean for that time point. Repeated measures ANOVA (RMANOVA) was used to analyze time (pre and post), intervention (GY versus PP), and interaction effects (intervention x time). Independent t-tests were used to analyze baseline data and percent change data between the groups, and an ANCOVA design was used to assess changes over time while controlling for baseline percent body fat differences.

Chapter 5: Results

5.1 Baseline Characteristics

Thirty participants were recruited for this study *via* signs posted at various locations at Brock University campus and *via* social media. Twenty-seven participants completed the 12-week intervention. One GY participant stopped exercise after 6 weeks due to injury (unrelated to the study). Two participants (1 PP and 1 GY) ended the study early (after 6 weeks of training) because they moved away from the university area. Post-testing was completed on all three of these subjects and their data were included in the analysis, with the exception of the injured participant who did not complete the 1-RM post-testing.

Table 5.1: *Baseline data for the two intervention groups.*

Variable	Baseline Value		Independent T-Test
	Greek Yogurt (GY)	Placebo Pudding (PP)	p-value
Height (m)	1.8 ± 0.0	1.8 ± 0.1	0.37
Structured exercise sessions/week	0.27 ± 0.5	0.17 ± 0.4	0.51
Physical Activity Level (sessions per week >15 minutes)	1.23 ± 1.2	1.0 ± 1.3	0.61
Body Mass (kg)	69.9 ± 9.6	69.7 ± 10.4	0.96
Fat-free Mass (kg)	60.1 ± 7.9	57.5 ± 6.9	0.63
Fat Mass (kg)	8.6 ± 4.0	12.2 ± 6.0	0.066
Body Fat (%)	12.3 ± 4.5	16.9 ± 7.2	**0.049**
Biceps Muscle Thickness (cm)	2.64± 0.4	2.75± 0.4	0.44
Quadriceps Muscle Thickness (cm)	3.81 ± 0.8	3.65 ± 0.7	0.55
Chest Press (kg)	81 ± 23	87 ± 18	0.62
Seated Row (kg)	84 ± 21	83 ± 17	0.88
Leg Extension (kg)	111 ± 24	124 ± 22	0.14
Leg Curl (kg)	79 ± 16	85 ± 15	0.26
1-RM Total (kg)	357 ± 80	379 ± 67	0.42
Calories (kcal/d)	2146 ± 407	1989 ± 398	0.27
Protein (g/d)	90.6 ± 15.2	85.7 ± 14.6	0.45
Protein (g/kg/d)	1.31 ± 0.32	1.25 ± 0.26	0.56
Carbohydrates (g/d)	246.1 ± 52.2	225.0 ± 54.9	0.28
Carbohydrates (g/kg/d)	3.46 ± 0.87	3.3 ± 0.89	0.48
Fat (g/d)	79.2 ± 18.0	79.9 ± 27.5	0.93
Fat (g/kg/d)	1.18 ± 0.27	1.15 ± 0.37	0.81
Calcium (mg/d)	699 ± 267	678 ± 225	0.88

Baseline values (displayed as mean ± SD). Statistical analysis was by independent t-test between both groups (GY and PP). Significance was set at p<0.05. RM = Repetition maximum.

5.2 Adherence to Study Supplements and Exercise

Trainers and study personnel ensured that the post-exercise supplement doses (of either GY or PP) were consumed in the laboratory, following training. This produced a 100% adherence rate for the post-exercise supplements. Bedtime and non-training day supplement doses were packaged by study personnel and taken home by participants at the beginning of each week. These supplements were consumed without direct supervision. Food diaries completed at week 12 indicated a 97% and 99% adherence rate for the intake of the unsupervised supplements for the GY and PP groups, respectively.

Exercise attendance was 31.6 and 30.1 out of 36 sessions for GY and PP groups, equating to an 88% and 84% adherence rate, respectively.

5.3 Strength

One outlier was removed from the dataset for chest press in the GY group, and none in the PP group. Voluntary 1-RM strength on chest press, seated row, leg extension, leg curl, and total strength were not significantly different between groups at baseline (Table 5.1). There was a significant main time effect for all 1-RM strength exercises ($p<0.001$). Significant interaction effects for the chest press ($p=0.026$), seated row ($p<0.001$), leg extension ($p=0.004$), and 1-RM total ($p<0.001$) indicated that the GY group gained more strength over time for these exercises than the PP group.

Table 5.2: *1-RM Strength measurements pre- and post-training.*

	Greek Yogurt (GY)				Placebo Pudding (PP)				RM-ANOVA		
	n	Pre	Post	Change	n	Pre	Post	Change	Time	Group	Interaction
		kg	kg	Δ		kg	kg	Δ	p-value	p-value	p-value
Chest Press	14	81 ± 23	103 ± 20	22 ± 12	15	87 ± 18	100 ± 20	13 ± 7	**<0.001**	0.82	**0.026**
Seated Row	15	84 ± 21	105 ± 23	21 ± 10	15	83 ± 17	93 ± 17	10 ± 6	**<0.001**	0.34	**<0.001**
Leg Extension	15	111 ± 24	150 ± 21	39 ± 15	15	124 ± 22	148 ± 27	24 ± 10	**<0.001**	0.51	**0.004**
Leg Curl	15	79 ± 16	92 ± 15	13 ± 8	15	85 ± 15	94 ± 17	9 ± 9	**<0.001**	0.42	0.22
1-RM Total	15	357 ± 80	455 ± 79	98 ± 37	15	379 ± 67	435 ± 76	57 ± 15	**<0.001**	0.96	**<0.001**

Strength values (displayed as means ± SD). Statistical analysis was by RM-ANOVA with time (pre and post) as the within factor and group (GY and PP) as the between factor. Significance was set at p<0.05. RM = Repetition maximum.

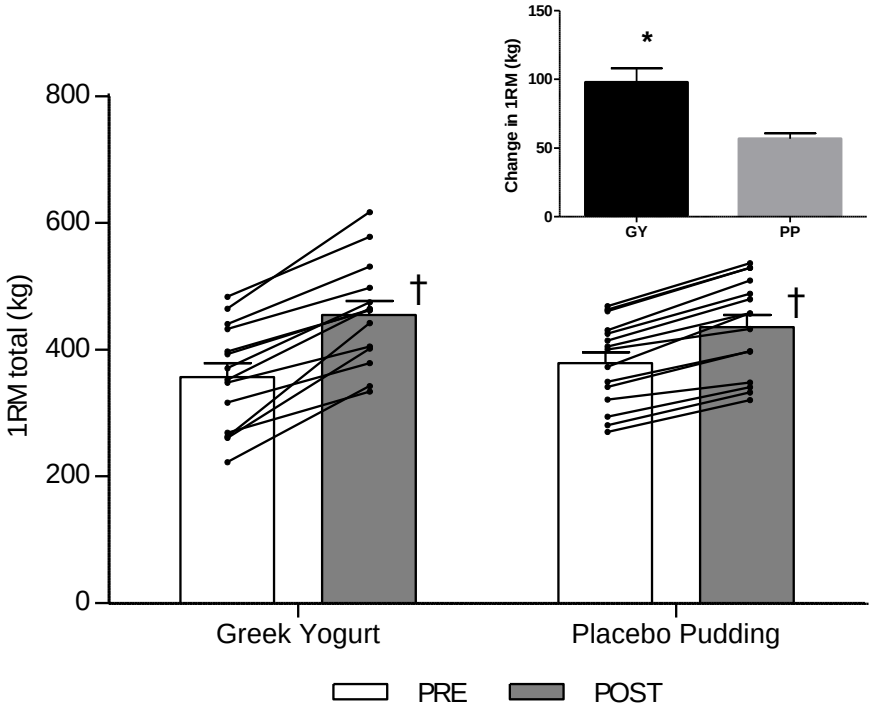

FIGURE 5.1—Total 1-RM strength before and 12 wk after RT and PLY in GY (n = 14) and PP (n = 15) groups. Individual pre and post responses are represented by the lines over the bars. The inset graph shows the change in total 1-RM strength from baseline. † Significantly different from Pre within the same group ($p<0.05$). *Significantly different from PP in the change from baseline in inset ($P<0.001$). Values are presented as mean ± SE. RM = Repetition maximum.

5.4 Muscle Thickness

One outlier was removed from the dataset for biceps muscle thickness in the GY group and none in the PP group. Post-intervention measurements of muscle thickness were only performed on 28 (n=14/group) participants because of technical issues with the ultrasound machine. Baseline muscle thickness of the biceps and quadriceps were not significantly different between groups (Table 5.1). Main time effects were present for muscle thickness of the biceps and the quadriceps ($p<0.001$). A significant interaction effect for muscle thickness of the biceps indicated that the GY group increased their average muscle thickness to a greater extent compared to the PP group ($p=0.004$).

Table 5.3: *Muscle thickness measurements analysed using ultrasonography of the biceps and quadriceps muscles pre- and post-training.*

	Greek Yogurt (GY)				Placebo Pudding (PP)				RM-ANOVA		
	n	Pre	Post	Change	n	Pre	Post	Change	Time	Group	Interaction
		cm	cm	Δ		cm	cm	Δ	p-value	p-value	p-value
Biceps	13	2.64± 0.4	3.1 ± 0.4	0.46 ± 0.3	14	2.75± 0.4	2.87 ± 0.5	0.12 ± 0.2	**<0.001**	0.70	**0.004**
Quadriceps	14	3.81 ± 0.8	4.47 ± 0.8	0.66 ± 0.4	14	3.65 ± 0.7	4.06 ± 0.7	0.41 ± 0.4	**<0.001**	0.27	0.14

Muscle thickness values (displayed as means ± SD). Statistical analysis was by RM-ANOVA with time (pre and post) as the within factor and group (GY and PP) as the between factor. Significance was set at p<0.05.

5.5 Body Composition

One outlier was removed from the dataset in the GY group and none in the PP group for each of the body composition variables. Body mass and fat-free mass were not significantly different at baseline (Table 5.1). A trend existed for baseline fat mass differences between groups (p=0.066). There was a significant difference for percent body fat at baseline between groups (p=0.049).

5.5.1 Fat-Free Mass

A significant main effect of time was observed for fat-free mass (p<0.001). A significant interaction effect for fat-free mass indicated that the GY group increased fat-free mass more than the PP group (p=0.046).

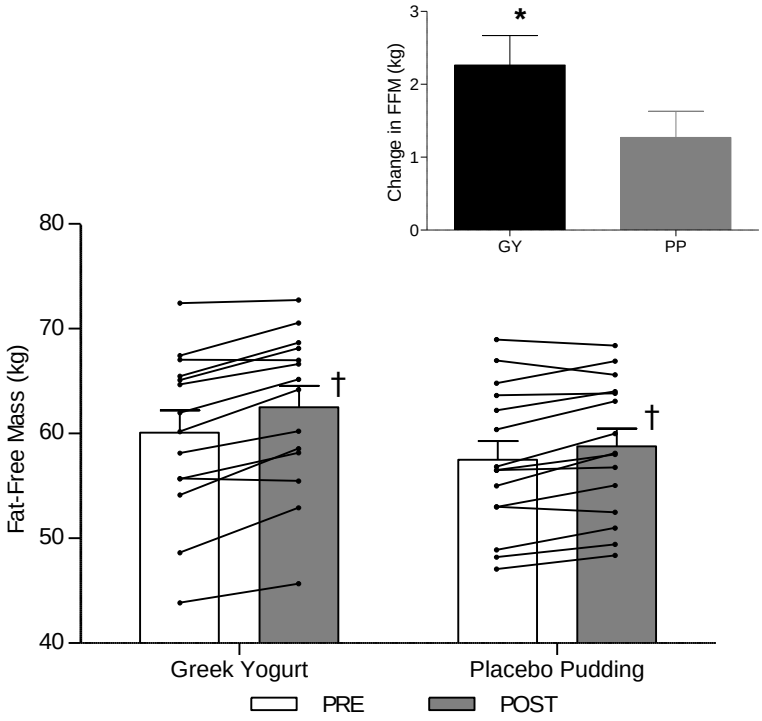

FIGURE 5.2— Fat-free mass before and 12 wk after RT and PLY in GY (n = 14) and PP (n = 15). Individual pre and post responses are represented by the lines over the bars. The inset graph shows the change in total fat-free mass from baseline. †Significantly different from Pre within the same group ($p<0.05$). *Significantly different from PP in the change from baseline in inset ($P<0.05$). Bars are presented as mean ± SE.

5.5.2 Fat Mass

There was a significant main effect of group for fat mass ($p=0.035$), with GY subjects having a lower fat mass than PP subjects regardless of time point.

5.5.3 Percent Body Fat

There was a significant main effect of group for percent body fat ($p=0.022$), with GY subjects having a reduced percent body fat than PP subjects. Because there was a significant difference in percent body fat between groups at baseline (Table 5.1), an ANCOVA was used with baseline percent body fat as a covariate, to assess the change in percent body fat between groups. The ANCOVA indicated that the GY group reduced percent body fat significantly more than the PP group ($p=0.048$).

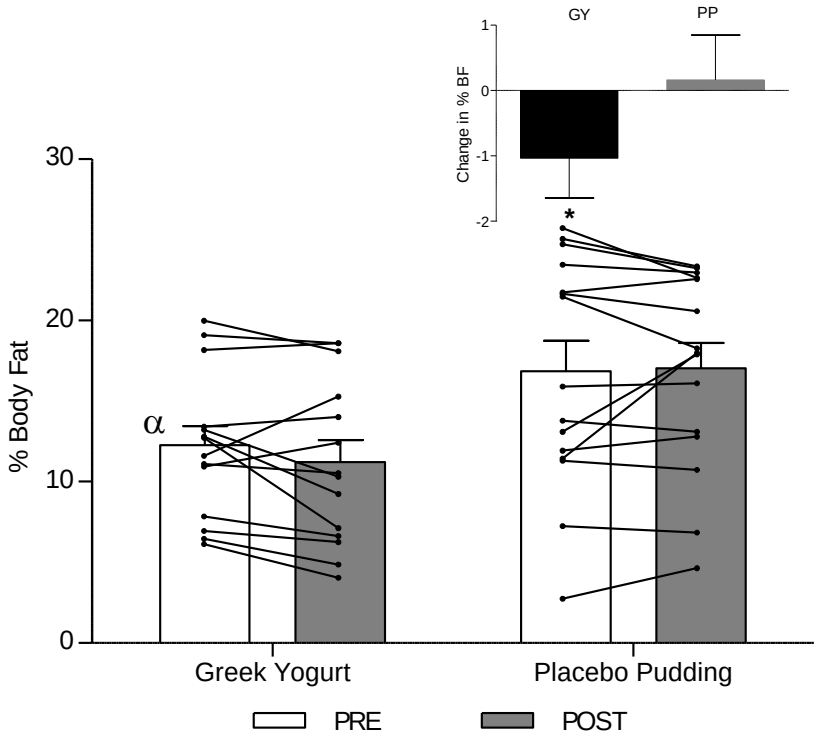

FIGURE 5.3—Total Fat-Free Mass before and 12 wk after RT and PLY in GY (n = 14) and PP (n = 15). Individual pre and post responses are represented by the lines over the bars. The inset graph shows the change in total fat-free mass from baseline. [α]Significantly different at baseline between groups ($p<0.05$). *Significantly different from PP in the change from baseline in inset as assessed by ANCOVA ($P<0.05$). Bars are presented as mean ± SE.

***Table 5.4**: Body composition measurements as assessed by BodPod pre- and post-training.*

	Greek Yogurt (GY)				Placebo Pudding (PP)				RM-ANOVA		
	n	Pre	Post	Change	n	Pre	Post	Change	Time	Group	Interaction
									p-value	p-value	p-value
Body Mass (kg)	14	69.9 ± 9.6	71.8 ± 9.5	1.9 ± 2.4	15	69.7 ± 10.4	71.4 ± 10.4	1.7 ± 2.0	**<0.001**	0.935	0.776
Fat-free Mass (kg)	14	60.1 ± 7.9	62.5 ± 7.6	2.4 ± 1.5	15	57.5 ± 6.9	58.8 ± 6.5	1.3 ± 1.3	**<0.001**	0.25	**0.046**
Fat Mass (kg)	14	8.6 ± 4.0	8.1 ± 4.4	-0.5 ± 1.8	15	12.2 ± 6.0	12.6 ± 5.4	0.4 ± 2.2	0.918	**0.035**	0.296
Body Fat (%)	14	12.3 ± 4.5	11.2 ± 5.1	-1.1 ± 2.2	15	16.9 ± 7.2	17.0 ± 6.1	0.1 ± 2.6	0.35	**0.022**	0.205

Body composition values (displayed as means ± SD). Statistical analysis was by RM-ANOVA with time (pre and post) as the within factor and group (GY and PP) as the between factor. Significance was set at p<0.05.

5.6 Nutrition

There were no differences in energy, protein, carbohydrate, fat, or calcium intake between groups at baseline (Table 5.1). Main time effects were present for energy (p=0.022), protein (absolute and relative to body weight; p<0.001), carbohydrate (absolute; p=0.003, and relative; p=0.009), and calcium (p=0.007) intake throughout the intervention (Table 5.5). Significant Interactions for protein intake (absolute and relative) and calcium intake indicated that the GY group had greater intakes than the PP group (<0.001). A significant interaction for carbohydrate intake (absolute and relative) indicated that the PP group had greater intakes than the GY group (p=0.002). There were no significant differences in fat intake throughout the intervention.

Table 5.5: *Daily dietary intake from food diaries for each group, at baseline and week 12.*

Nutrient intake per day	Greek Yogurt (GY)			Placebo Pudding (PP)			RM-ANOVA		
	n	Baseline	Week 12	n	Baseline	Week 12	Time	Group	Interaction
Energy (kcal)	14	2146 ± 407	2207 ± 345	15	1989 ± 398	2303 ± 588	**0.022**	0.83	0.11
Protein (g)	13	90.6 ± 15.2	124.8 ± 13.4	15	85.7 ± 14.6	85.9 ± 19.9	**<0.001**	**<0.001**	**<0.001**
Protein (g/Kg)	13	1.31 ± 0.32	1.74 ± 0.31	15	1.25 ± 0.26	1.22 ± 0.27	**<0.001**	**0.007**	**<0.001**
CHO (g)	15	246.1 ± 52.2	242.2 ± 55.2	14	225.0 ± 54.9	283.3 ± 55.2	**0.006**	0.57	**0.002**
CHO (g/Kg)	13	3.46 ± 0.87	3.38 ± 0.71	14	3.3 ± 0.89	4.04 ± 0.9	**0.013**	0.416	**0.002**
Fat (g)	15	79.2 ± 18.0	78.4 ± 18.6	15	79.9 ± 27.5	84.9 ± 35.7	0.57	0.68	0.43
Fat (g/Kg)	15	1.18 ± 0.27	1.11 ± 0.26	15	1.15 ± 0.37	1.19 ± 0.46	0.81	0.84	0.32
Calcium (mg)	14	699 ± 267	1069 ± 243	14	678 ± 225	585 ± 211	**0.007**	**0.003**	**<0.001**

Nutrient intake values (displayed as mean ± SD). Statistical analysis was by RM-ANOVA with time (pre and post) as the within factor and group (GY and PP) as the between factor. Significance was set at p<0.05.

5.7 Percent Change

Percent change was calculated for each variable using the equation: ((post-pre)/pre)x100. Independent T-Tests (Table 5.6) revealed a greater percent change decrease in fat mass and percent body fat in the GY group compared to the PP group (p=0.042 and p=0.038, respectively). Similar to the RM-ANOVA results, percent change for the biceps muscle thickness and 1-RM strength measures (except the leg curl) were greater for the GY group compared to the PP group (Table 5.6).

Table 5.6: *Subjects percent (%) change for both groups, from pre to post intervention.*

Variable	Greek Yogurt (GY)		Placebo Pudding (PP)		Independent T-test
	n	%	n	%	p-value
Body Mass (Kg)	15	2.4	14	2.0	0.77
Fat-free Mass (Kg)	15	3.9	15	2.3	0.11
Fat Mass (Kg)	14	-11.1	14	5.8	**0.042**
Body Fat (%)	14	-13.2	13	-1.1	**0.038**
Biceps Muscle Thickness (cm)	14	16.4	13	7.1	**0.026**
Quadriceps Muscle Thickness (cm)	14	15.0	14	13.0	0.67
Chest Press (Kg)	13	28.3	15	15.4	**0.030**
Seated Row (Kg)	13	23.7	14	11.7	**0.002**
Leg Extension (Kg)	14	11.7	14	20.9	**0.006**
Leg Curl (Kg)	13	14.6	14	12.8	0.62
1-RM Total (Kg)	13	26.8	15	15.1	**0.003**

% change values (displayed as means). Statistical analysis was by independent t-test between groups (GY and PP). Significance was set at p<0.05.

Chapter 6: Discussion

6.1 General Discussion

Our data demonstrate that the consumption of plain, 0% fat Greek yogurt following resistance and plyometric exercise (600 g on training days, 300 g on non-training days) increased most measures of strength, biceps muscle thickness and fat free mass and reduced fat mass more than an isoenergetic, carbohydrate-based placebo pudding consumed at the same time points. This study is the first to use Greek yogurt in this context to demonstrate such an effect with resistance exercise. We believe it is important to investigate the combined effect of GY and exercise as this is a practical representation of a healthy lifestyle change.

Our research supports previous findings where milk supplemented post-RT was shown to increase strength and lean mass greater than carbohydrate (2,3). We chose voluntary 1-RM strength as one of our primary outcome measures. Strength is an important functional measure and can be used as a surrogate for muscle size and lean mass as these variables are highly correlated (172). Also, there is a strong positive relationship between strength and muscle size (173), and our data demonstrated this ($R=0.61$, $P=0.001$). There was a time by group interaction effect for the chest press, seated row, and leg extension exercises as well as the 1-RM total indicating that the GY group increased strength more than the PP group. Due to the relatively fast adaptations incurred within novice exercisers (48,50), protein intake of approximately 1.6 g/kg/day may be necessary for individuals new to RT to augment morphological adaptations which are necessary to facilitate optimal strength gains (71–73). The justification for the increased protein recommendation of 1.6 g/kg/d is due to a higher rate of muscle protein synthesis in novices (48) and a reduced efficiency of protein utilization compared to trained individuals (174). GY supplementation yielded an increase in subject's protein intake to 1.74 g/kg/d versus PP subjects who were consuming protein below the recommended level (for individuals new to

RT) at 1.22 g/kg/d (71). These data suggest GY supplementation enabled subjects to increase chronic protein intake in excess of the recommended 1.6 g/kg/d for individuals involved in frequent RT and therefore likely facilitated greater strength adaptations than the PP group (71,72,108). Initially, during a RT program strength gains are typically the result of neurological adaptations such as enhanced inter-muscular coordination (175). However, to continue to develop muscular strength, morphological adaptations are necessary. These adaptations include increasing muscle cross sectional area by increasing contractile proteins, altering tendon and connective tissue, changes in fibre type and hyperplasia, all of which require additional dietary protein (175).

Research indicates that novice exercisers can experience 7.9% strength and 8.8% muscle-CSA increases after just 1 month of training (48). The present study showed that total 1-RM strength increased by 27% in the GY group compared to 15% in the PP group after 12 weeks (3 months) of training. These results suggest that chronically elevated daily protein intakes (from GY) combined with RT can promote significantly greater gains in muscular strength compared to the consumption of daily protein at lower levels (1.22 g/kg) but still 53% above the current RDA of 0.8 g/kg/d. Our research did demonstrate a main effect of time for all voluntary 1-RM exercises plus the 1-RM total (Table 5.2, Figure 5.1). This was expected because a training stimulus alone is likely sufficient to increase strength following 12 weeks of exercise (175). Our research is consistent with other chronic (minimum 10 weeks) training studies in young, untrained males which demonstrate that increased protein intakes optimize strength adaptations during a RT program (2,121,125,130,175).

Muscle thickness was another of our main outcome measures. The data from the present study revealed a significant main effect of time for biceps and quadriceps muscle thickness following the intervention. This can likely be attributed to the effectiveness of the exercise program in stimulating muscle hypertrophy. The data also indicated that habitual consumption of GY while undergoing regular RT (and PLY) training yielded greater increases in biceps brachii muscle thickness compared to the

consumption of the PP. These findings support similar research in milk (2,16) and isolated dairy protein (whey and casein) supplementation (121,125,129) where greater increases in muscle size occurred compared to a placebo following RT in previously untrained, young, male subjects. RT causes metabolic and mechanical stress to the muscle which signals MPS to occur (176,177). Once this stimulus has occurred, hyperaminoacidemia is required to facilitate the incorporation of AAs into the muscle to make new myofibrillar proteins (178). If this process is consistently repeated, like our study design intended, total muscle cross-sectional area (CSA) can be increased (179). We measured muscle thickness, a surrogate for muscle CSA, *via* ultrasonography (180). Currently, the gold standard for measuring muscle size is a CSA determination *via* magnetic resonance imaging (MRI) (181). However, MRI technology is expensive and was inaccessible for this study. Ultrasonography is another method for measuring muscle size and has been shown to be reliable and valid when compared to MRI (181,182). Research indicates that as long as the tester and body position remains consistent, results are reliable (183). Our imaging measures indicated a significantly greater increase in biceps muscle thickness after consumption of GY versus PP.

A main effect of time was present for total body mass following the intervention in both groups. This may be explained, in part, by the increase in fat-free mass (FFM), but also the increase in energy intake experienced by both groups. Importantly, and the last of our main outcome measures, the GY group had a more favourable (increase in FFM and decrease in %BF) body composition change than the PP group (Figure 5.4). All the body mass they gained was FFM and they lost fat mass (100% and -26%, respectively), whereas the PP group gained both FFM and fat mass (76% and 24%, respectively). A significant main effect of time existed for FFM indicating that both groups increased FFM, but an interaction effect was also present, indicating that the GY group gained significantly more FFM than the PP group. Since the training program was consistent for both groups, this likely reflects the increased protein intake and any other specific features inherent in GY that could contribute to this effect. Of

note, our measure of FFM includes organ, bone, water, connective and muscle tissue, therefore it does not measure muscle mass alone. However, it is likely that the majority of measurable gains in FFM would be muscle mass since other major components, like bone, can take up to 6 months to detect significant mass changes (184). Nonetheless, research shows that RT in young adult males is associated with increasing bone and connective tissue mass (126), but the magnitude of change in these compartments is smaller. In terms of our data, the FFM changes corroborate the robust strength increases and increases in muscle thickness (and differences between groups), all of which reflect the assertion that our intervention likely increased the accretion of muscle tissue.

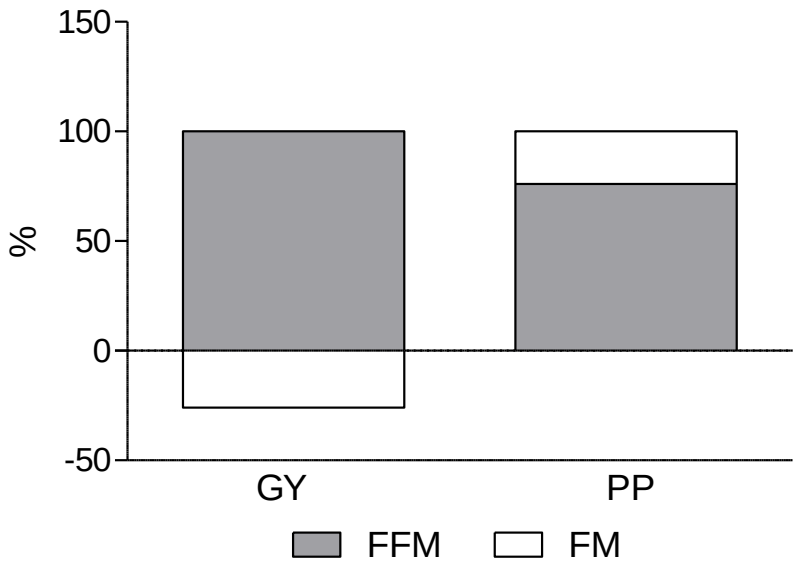

FIGURE 6.1 – Fat mass and Fat-free mass expressed as a percent of total mass gained (and lost) during the intervention for the GY and PP groups (GY= 14, PP= 15).

The results of the current study are comparable to the results of two other studies investigating milk supplementation with RT in previously untrained males (2,17). The results from our study and the Hartman et al., (2007) (2) study showed positive effects of dairy on strength compared to a CHO placebo, whereas Rankin et al., (2004) (17) did not show any difference between chocolate milk and a

CHO placebo. Our results also showed that GY facilitated an additional increase (over the PP group) of 1.1kg of FFM which is in-between the 1.5kg of fat and bone free lean mass (FBLM) previously observed with a milk intervention (2), and the 0.8 kg FBLM observed with chocolate milk (17). However, the FBLM results from Rankin et al., (2004) were not significantly greater than the CHO control, as it was in the present research and in Hartman et al., (2007). There may be several reasons that there was no significant difference in the Rankin study. For instance, the addition of chocolate milk (Protein: 17 g/serving, 1 serving/d, x3d/wk) only increased habitual protein intake to 1.3 g/kg/d, whereas their control group was consuming 1.2 g/kg/d, the study design was only 10 weeks in duration (RT 3 d/wk), it may have been underpowered (total subjects; n=19) and their training protocol progressively reduced volume (and increased %1-RM) which limits variables that favour lean mass accretion, such as time under tension (17). Our study was of longer duration, which may have offered more time for greater adaptations. It had a larger sample which may have reduced the SD and increased our power to detect between-group differences. Finally, our training prescription promoted FFM accretion. All of which may have led to our significant findings. Perhaps an explanation for greater increases in FFM mass in the Hartman study can be attributed to the increased frequency and volume of the training protocol, as subjects underwent RT 5d/wk, compared to only 2d/wk (plus PLY 1d/wk) in our study. Research indicates that increasing frequency (134,151,185) and total volume (186–188) is associated with greater training-related adaptations.

A major difference between the two intervention groups was the amount of protein provided by our supplements and hence the amount of protein consumed during the intervention. GY provided 20 g of protein/200 g serving, whereas PP provided 0 g protein. The results from the food diaries at week 12 were consistent with our supplementation protocol as there was an increase in absolute (g/d) and relative (g/kg/d) protein intake from baseline in those consuming GY, while those consuming PP experienced similar increases in carbohydrate and not protein (Table 5.5). Therefore, the GY group was

chronically consuming a higher level of protein during the intervention, which likely contributed to the observed divergent results in our outcome variables favouring the GY group. Beneficial characteristics of GY such as its satiating effect (92,189,190), probiotic cultures (19,21) and micronutrients (e.g. calcium) (18,68) may have offered additional benefits to digestive (23,30,31) and bone (191–193) health, although these were not directly measured in this thesis.

In our study, we provided subjects with 2x20 g (i.e. 40 g) of dairy protein (from Greek yogurt) over 1 hour post-exercise. Research indicates that this would provide a sufficient amount of protein to facilitate an anabolic environment and maximally stimulate MPS (72,73,108). In young persons, 20 g doses of protein are just as effective at stimulating MPS as 40 g doses at rest (96,105), and 0.24 g/kg/meal (also at rest) is sufficient to stimulate myofibrillar protein synthesis (150). A recent review proposed that this dose should be greater, especially when the protein source is part of a whole food, is slower digesting and when consumed in the presence of other macronutrients which may further delay absorption and enhance AA utilization (73). A review by Schoenfeld & Aragon in 2018 suggests 0.4 g/kg/meal may be the optimal dose for exercising individuals to maximize anabolism (73). For this reason, we formulated our doses accordingly following training (2x20g doses within 1 hour post-exercise) to help optimize MPS by providing the recommended dose in a whole-food supplement for most subjects. Based on the different 0.24 g/kg/dose and 0.4 g/kg/dose recommendations, for a 70 kg individual, this corresponds to 16.8 g and 28 g of protein per dose/bolus respectively. Our dose (20 g) falls between these two. We also used this post-exercise dosing regimen to mimic the successful designs of the Hartman (2), and Josse (3) studies with milk. Our participants also consumed GY prior to sleep (200 g on training days, 150 g on non-training days) in efforts to minimize MPB and maintain a positive net protein balance during sleep, since sleep tends to be a fasted period in which protein balance naturally favors breakdown (124,194). An acute study by Res et al., (2012) gave participants 40 g of intrinsically labelled casein protein prior to sleep 3 hours following a RT bout (57). Blood samples

collected throughout sleep revealed that casein was effectively digested and absorbed, resulting in increased circulating AA levels throughout sleep (7.5 hours). This was accompanied by significantly greater whole body protein synthesis rates and an improved net protein balance versus the placebo (57). The study by Res et al. (2012) preceded a chronic training study by Snijders et al., (2015) which showed that daily consumption of 27.5 g of casein protein prior to sleep was able to augment muscle mass and strength gains following 12 weeks of RT (125). This may be, in part, due to the ability of casein to attenuate MPB which facilitates greater lean mass gains due to the preservation of muscle tissue (125). However, it is important to note that in this study, subjects consuming casein before sleep were also habitually consuming more overall protein (1.9 g/kg/d) than the placebo (1.3 g/kg/d) group throughout the intervention which may be the reason for the greater adaptations. GY primarily consists of casein protein, and our study protocol also had GY subjects consuming GY prior to sleep, therefore a similar mechanism yielding subsequent FFM gains may have occurred in our study.

Limited research exists on the postprandial absorption rate and plasma AA response of GY. However research with intrinsically labelled casein protein indicates that absorption is even slower when consumed in a whole food (i.e. milk) matrix versus isolated micellar casein (195,196). This research also discovered that a higher proportion of casein consumed from milk was incorporated into skeletal muscle than when consumed as isolated casein, suggesting that the presence of other nutrients may influence and further delay absorption, which ultimately increases AA utilization by muscle tissue (196). Additionally, this research analyzed the absorption properties of whey and casein when consumed together in milk using stable-isotope tracer methodology and indicated that at 220-260 minutes, blood concentration of AAs from casein was significantly greater than from whey (196). Considering that GY likely has a greater ratio of casein to whey than milk (based on the manufacturing process of removing the liquid-whey from GY (10,24)), is more acidic (197), and exists in a semi-solid food matrix (25), all of these factors could attenuate digestion and subsequent absorption rates such

that GY would elevate blood-AA concentrations for a longer duration than milk. For this reason, GY may be more beneficial at attenuating MPB than milk or micellar casein alone, but we cannot confirm this with our research. Nonetheless, as detailed above, we provide subjects with a pre-sleep protein dose in efforts to minimize MPB and provide a sustained influx of AA to maintain a positive (or less negative) protein balance overnight.

Recent research on skim milk suggests that increasing its acidity may increase the absorption rate of AAs due to anti-coagulation effects (198). Skim milk with increased acidity (pH= 4.1) was absorbed quicker than regular skim milk (pH=6.9) and subsequently increased plasma essential AA, plasma leucine levels and MPS significantly greater at 30 minutes post-ingestion (198). The acidity of fat-free plain GY (pH= 4.35-4.65 (199)) closely resembles that of the acidic skim milk condition mentioned above. However, milk and GY have distinct differences such as their viscosity and differing dairy protein ratios which may affect gastric emptying and nutrient absorption. More research is needed regarding the absorption and appearance rates of plasma AAs from GY to better establish how all of its unique properties effect digestion, absorption and incorporation into muscle tissue.

The GY group had significantly less percent body fat at baseline compared to the PP group (GY: 12.3%, PP: 16.9%). The rate at which body fat is lost and the amount of initial body fat are inversely related (185). This means that body fat becomes increasingly more difficult to lose as an individual becomes leaner. For our analysis, we opted to control for the statistical baseline difference in body fat percent. Using an ANCOVA model, with baseline-percent body fat as a covariate, the analysis showed a significantly greater reduction in percent body fat in GY subjects compared to PP subjects. Without accounting for baseline differences, our data demonstrated that the GY group still lost more fat mass (Table 5.6) and % body fat (Table 5.4) than the PP group. The superior effect of dairy, as seen in our study and others (2,3), on fat mass may be due to the increased calcium consumed by participants within these dairy groups. Research indicates that high dairy and calcium diets may attenuate adipocyte

growth and thus body weight gain during periods of increased energy intake (68). A meta-analysis concluded that high calcium diets are believed to inhibit lipogenesis and promote lipolysis, lipid oxidation and thermogenesis thus having a meaningful effect on weight control (99). Research also indicates that dairy sources of calcium are able to achieve these results more effectively than supplemental sources of calcium due to the combination of other bioactive compounds, such as angiotensin-converting enzyme which has purported anti-obesity effects (68). Three or more servings of dairy products/day has been associated with significant reductions in fat mass with (100) and without (68) energy restriction. Food diaries from our study indicated that GY supplementation increased calcium intake to 1069 mg at week 12, compared to 585 mg for PP subjects (Table 5.5). Research by Boon et al., suggests that a threshold for calcium intake improving body composition exists at 800mg/day (200). GY was able to habitually increase calcium intake beyond this level and may have contributed to the observed reduction in percent body fat seen within the GY group. It was also suggested that individuals with habitually low calcium intake benefit more in terms of fat loss from increasing calcium levels than those with habitually high levels (99). This may have conferred additional benefit in our study as the GY group was only consuming 699 mg of calcium/day prior to the intervention. Habitual yogurt consumption has also been associated with better long-term body weight control (94,201). These findings are partly attributed to the satiating characteristics of yogurt which relate to the effects of dairy protein and calcium on appetite control (189,202,203). Despite a significant time effect for energy intake in the current study indicating an increase in energy, the GY group was able to attenuate an increase in % body fat more than the PP group (Figure 5.3, Table 5.4). In fact, the GY group lost significantly more fat mass and % body fat than the PP group expressed as the percent change from baseline. This could perhaps be partly explained by the positive effect of calcium on lipolysis and fat oxidation (68,98,101,102,204–206).

To our knowledge, the only other study involving yogurt consumption and RT showed no greater fat mass or body composition changes between groups (regular yogurt, versus isoenergetic/isonitrogenous supplement [Accel Gel], versus isoenergetic supplement without protein [Clif Shot]) (13). However, this study used female participants, the length of the intervention was only 8 weeks, and the yogurt (regular, not Greek) contained 5 g of protein per serving (3 servings/day) (13). The length of the intervention may have been too short, and the amount of protein (15 g/daily) may have been insufficient to see a difference between groups. The yogurt supplementation only increased habitual protein intake to 1.1 g/kg/d compared to 1.0 and 0.9 g/kg/d in the other two groups. The yogurt group in this study was also consuming more energy than the other groups which may have negated a potential effect of yogurt on fat loss. Although there was a significant effect of time in the present study for energy intake, the energy intake of the GY group did increase less than the PP group.

High protein GY has demonstrated the ability to reduce appetite and energy intake in subsequent meals compared to lower protein snacks and snack-skipping (18). Although appetite was not measured in our study, the GY group did consume less daily energy (2207kcal compared to 2303kcal at week 12) than the PP group, although the difference between the groups was not significant. The rationale for choosing plain (unflavoured) GY as opposed to flavoured GY was due to its higher protein content and reduced sugar content. The GY used in the present study contains 20 g of protein and 5 g of sugar per 200 g serving compared to (e.g.) cherry-flavoured GY, which contains 16 g of protein and 24 g of sugar and an additional ~70kcal per equal serving. Yogurt with higher protein to CHO (plain) ratios have been shown to improve post-meal glucose control and satiety compared to yogurt with honey, milk, and orange juice (95). Yogurts with higher protein content (24 g per serving) have been shown to reduce hunger and delay subsequent eating greater than lower protein (5 g and 14 g per serving) yogurts (94).

The supplementation in our study provided each group with 330 calories per training day (3 doses of supplement) and 165 calories per non-training day (1.5 doses of supplement). Our data show that both groups did not completely compensate their habitual diets for the added supplementation (albeit the GY group did consume less additional energy than the PP group) which caused them to significantly increase their energy intake from baseline. The GY group only increased their habitual energy intake by 61 calories at week 12, compared to 314 calories in the PP group. Although this increase in energy was not statistically significant (between the groups), the consumption of 300+ kcals/d over time is arguably more physiologically significant than an increase of 61 kcals/d (207). For example, a 6 month study that replaced caloric beverages with noncaloric beverages, a straight-forward strategy to reduce energy intake, resulted in 2.5% weight loss (207). In our study, subjects were also exercising vigorously 3x per week thus expending energy which may have helped to better facilitate energy balance by decreasing the impact of the added energy. Despite this, there were still significant differences in body composition between groups such that all the increase in body weight in the GY group was FFM (100% FFM and -26% FM), whereas this was not the case in the PP group (76% FFM and 24% FM) (Figure 5.4). This difference depicts a more favourable overall body compositional change in the GY group versus the PP group and may relate to the fact that the added energy in the GY group was predominately protein compared to CHO in the PP group.

Research has shown that the consumption of 100 g of CHO following exercise has the ability to increase net protein balance compared to a non-caloric placebo (128). For this reason, when assessing the effect of protein (as yogurt) post-exercise, it is important to compare it to an energy-matched CHO placebo. This has been done in several related studies (2,3,121,130–132,179,13,17,29,70,74–77). An interesting and novel aspect of our study was the use of a semi-solid CHO placebo which closely resembled the viscosity of GY. Much of protein supplementation research focuses on liquid, isolated protein supplements and thus liquid, CHO-based placebos (2,3,130–132,208,17,29,74–77,121,129). This

may be to accelerate gastric emptying and promote the quick appearance of AA in the blood to facilitate MPS. However, there are other important elements to consider when improving body composition. For example, research indicates that solid CHO is more satiating than liquid CHO and is able to attenuate over-eating behaviours (209). This may have further implications for fat mass loss with GY. In this study, we designed an energy-matched, protein-void, CHO placebo which closely resembled the consistency and texture of GY to help control for the satiating effects of semi-solid foods that require mastication as opposed to fluid drinks (210). The CHO naturally present in GY (9 g of CHO per 200 g serving) may also have a beneficial role in lean mass accretion and the promotion of a positive protein balance (128). First, the probiotics within yogurt assist in breaking down lactose into its constituent monosaccharides galactose and glucose (60), which are absorbed rapidly (211). Second, CHO absorption increases circulating insulin levels which further promotes an anabolic environment for MPS (128) while subsequently inhibiting MPB (212). However, research has shown that CHO may only be necessary when protein intake is inadequate because protein is also insulinogenic. A study providing 25 g of whey protein plus 50 g of maltodextrin did not further effect MPS or MPB more than 25 g of whey alone (213). Importantly, GY is predominately casein protein (not whey), which is absorbed more slowly and therefore the effect/impact of CHO on protein balance following RT may be more important when the delivery of AA to the muscle is delayed due to the delayed insulin secretion (based on the absorption kinetics of the ingested protein). However further research is required to investigate this theory.

6.2 Strengths

Our study had several strengths. The use of only one tester for all subject pre- and post-testing was a strength as this minimized inter-tester variation. All supplementation was prepared by the same individual as well to ensure consistency. Deciding to keep the contents of the PP discreet was also a strength of the study, as this may have facilitated our high supplement adherence rates (because the participants may have thought the PP contained different beneficial bioactives). Trainers involved in the

study were also unaware of the contents of the PP to prevent bias. Exercise training was kept consistent between participants and groups as all trainers in the study went through the same orientation sessions and many of the trainers trained several participants from both groups. Another strength was that we compared GY to an isoenergetic supplement of the same consistency devoid of protein. Evidence suggests that just the provision of calories post resistance exercise can facilitate an anabolic effect (127,128). The goal of our study was to assess GY in its entirety but also as a vehicle for the delivery of protein and nutrients that support positive body composition change. Thus, if we did not at least provide energy at the same intervals, our study would be flawed and biased (in our favour). A final global strength of our study is that it is the first to report a positive effect of GY with exercise on a comprehensive set of outcome variables relating to physical (musculoskeletal) health (including strength, body composition and muscle thickness) which allows us to definitively and robustly assert GY's beneficial role within this context.

6.3 Limitations

Our study also had limitations. Subjects were not blinded to which supplement group they were in. Blinding is notoriously difficult to achieve in nutrition studies (214–216). However, we did conceal the contents of the PP from our subjects (and trainers). Furthermore, we called the PP the "study-designed supplement" which kept its contents and purpose within the study discreet. Although it was a strength to have one person perform all the subject testing, this person was aware of the randomization which could be a weakness (due to the potential for unintentional bias). A weakness in our study may be that we did not use state-of-the-art measurement tools, such as DXA, for body composition determination (217–219). We used the Bod Pod which is only able to measure fat and FFM (using a 2-compartment model), and therefore is unable to give a specific measure of muscle mass. However, the Bod Pod is considered a reliable method for measuring body fat in normal weight populations compared to DXA (170,171). Although still accurate, ultrasonography to measure muscle thickness (which is not a direct

measure of muscle CSA) and the Bod Pod to measure body composition (which is not a direct measure of muscle mass) are not the gold standards in their respective areas and may have lacked sensitivity to detect small changes. Another limitation may be that since subjects were initially untrained, they may have experienced a learning effect on the 1-RM exercises which may partly explain the increased strength during the post-testing. However, this would have been consistent for all participants regardless of group and cannot explain the divergent results in favour of GY.

6.4 Implications

The current research is congruent with existing literature indicating that protein supplementation that increases an individual's daily protein intake to 1.4-1.6 g/kg/d is optimal to support increased strength, muscle size, and improved body composition while in a RT program (71–73). This study is the first to investigate this phenomenon using GY; a solid, whole food, protein-rich, product, as opposed to isolated AA, protein supplements or liquid milk. Hartman et al., and Josse et al. have previously demonstrated positive effects of RT with milk in young men and women, respectively (2,16). Our results indicate that individuals novice to RT can make significant physical adaptations by consuming GY. In addition, GY is nutrient dense and its consumption can improve overall diet quality and offer other health benefits such as improved digestive (23) and bone health (192,193,220). GY is versatile and may serve as a vehicle for the consumption of other healthful foods which would also improve diet quality. Yogurt consumption may increase consumption of fruits and cereals which provide antioxidants, polyphenols and prebiotic fibers. Together these properties of yogurt may provide multiple effects that benefit musculoskeletal, digestive, cardiovascular and metabolic health (26).

Our study provides further evidence that GY could serve as a practical and functional food to increase dietary protein and calcium as well as other important nutrients, and thus may be useful in this population. Obese and overweight individuals may also benefit from consuming GY as part of a healthy

diet program due to the effects of protein on satiety and lean mass preservation (especially important during an energy-deficit (90,221–223)) and calcium's role in reducing fat mass (11,98). Our data show that 1.74 g/kg/d was more effective at increasing strength, muscle thickness and fat-free mass than 1.22 g/kg/d. This supports current position stands on protein recommendations for novice resistance exercisers indicating that 1.6 g/kg/d may be a more optimal level of protein to facilitate positive training adaptations (71,72,108). In terms of the current RDA for protein (0.8 g/kg/d), our data indirectly demonstrate that young healthy males not only habitually consume well above the RDA (~50% above) but that this level of intake is insufficient for individuals involved in RT. Lastly, our data also provide support for a mixed-modality approach to training as combining RT and PLY significantly improved strength, muscle thickness, and fat free mass in both groups. This combination may more closely resemble the type of physical activity of the general population (part RT, part PLY/sport/high intensity exercise). Coaches and dietitians can use these results to recommend GY to athletes or any individuals beginning a mixed exercise program with the goals of increasing strength and improving body composition.

6.5 Future Directions

The properties of and nutrients in GY may also be beneficial for bone health. Increasing bone strength and density in early adulthood can reduce the risk of fractures and osteoporosis later in life (224). GY could also be beneficial for the older adult who, as a population, are characterized as under-consuming protein (225,226). Older adults are also less sensitive to the anabolic effects of protein and therefore require more to maintain their muscle and bone health with age (227,228). Additionally, GY should be further investigated in overweight populations for its effects on body composition and satiety (11). Future research could investigate GY consumption in the context of digestive health. A healthy digestive system and microbiome have been associated with general health and healthy aging due to their impacts on immunology and metabolism (62). Future research should focus on strategies to

incorporate GY into the diets of older persons, especially breakfast meals as this is often a CHO-dominated meal low in protein and research suggests adding 20-30g of high quality protein to each meal should be strived for in efforts to achieve healthy aging (229). Research could also directly compare GY against other popular protein sources such as milk or whey in a similar intervention study with the same outcomes. Lastly, future research should also characterize the postprandial AA profile and absorption kinetics of GY, specifically noting the leucine and BCAA content, as these factors are important for MPS and overall protein balance (135,230).

6.6 Conclusion

Habitual GY consumption compared to a CHO-based pudding during a 12-week RT/PLY program increased strength and muscle thickness while improving body composition in young healthy men who are new to resistance exercise. Based on our results, GY should be consumed, especially by individuals beginning a RT program, to increase protein intake and to facilitate favourable training adaptations. GY may be able to increase overall health in several ways that extend beyond muscular benefit while attenuating disease risk.

7. References

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on the training volume of male athletes. Physcial Educ Sport. 2007;5:37–46.

8. Appendix

8.1 Exercise Training Program – variables to consider

8.1.1 Duration of Exercise

Chronic RT has been shown to promote increases in strength and muscle hypertrophy in untrained subjects (231). A study was designed to measure the process of skeletal muscle growth in untrained, young adult males who participated in an 8 week (2 RT sessions, 1 test session/week) RT protocol (232). Participants underwent weekly pQCT (peripheral quantitative computed tomography) measures to assess muscle cross-sectional area (CSA). The study found a significant mean increase in the thigh of 5 cm^2 (3.46%) after only 1 week of training (3 sessions). However, researchers hypothesized these results may be due to muscular edema, as a limitation of pQCT is the inability to distinguish between muscle tissue and intramuscular fluid. To account for this potential edema, researchers used week 1 as the new baseline and still found significant increases in muscle CSA after 3 weeks. Overall, the study showed that 8 weeks of RT (3 sets/exercise to failure) significantly increased muscle CSA from baseline by 13.9 cm^2 (9.6%). Researchers concluded that significant muscle hypertrophy can occur in 3-4 weeks following RT (232). Another study indicated that muscular hypertrophy can occur after 20 days of RT (3x/week) (233). This study only used bilateral leg extension training 3 times a week and assessed quadriceps muscle CSA. Another study saw no hypertrophic effects after 5 weeks of training (3x/week), however, this study assessed both males and females together, and the training was primarily strength-focused (5 sets of 6 repetitions of unilateral knee extensions) which could potentially be the reason no muscular size adaptations occurred during the relatively short training period (234). Additionally, no dietary information was reported in any of these studies, which could have cofounded the results pending protein intakes. A review by Pasiakos et al., (2015) indicated that studies involving protein supplementation (versus placebo) and RT which are 8 weeks and shorter have a tendency to show no

group by time effects, however studies which are 9 weeks and longer tend to display significant differences (161).

8.1.2 Intensity of Exercise

Intensity may be the most influential variable in regards to inducing muscular hypertrophy (235). Intensity can be further divided into load/weight and repetitions, each with their own optimal range for producing hypertrophic responses. Loads should be at least 65% of 1 repetition maximum (1 RM; which is a strength measure of the weight that can be moved over the full range of motion for one complete repetition) in order to sufficiently stimulate muscular hypertrophy (236). Moderate rep ranges between 6-12 appears to be the optimal range to promote hypertrophy (237). A moderate rep range provides an optimal balance between sufficient mechanical tension and metabolic damage necessary to trigger muscle hypertrophy (136). The current literature on training intensity necessary to elicit the optimal hypertrophic response is unclear, as more recent research has indicated that sets taken to volitional failure may be more important than reps or load (153,154,156,238). Burd et al. (2010) directly tested this phenomenon and showed that a low load (30% 1-RM; leg extension) taken to volitional failure was able to elicit a similar acute MPS response 4 hours post-RT as a high load (90% 1-RM) and work matched loads (30% 1-RM work matched to 90% 1-RM). However at 24 hours post-RT, the low load (30% 1-RM to failure) group experienced a greater MPS response than the other two groups (153). Similar research supported these findings with the bench press (155). It is thought that the increased time-under-tension, even with lighter loads (i.e. 30% of 1-RM), along with increased metabolic stress (from completing more total work than 90% 1-RM) is responsible for the enhanced hypertrophic effects (239). Burd et al., (2011) conducted further research indicating that unilateral leg training to failure (either 90% or 30% 1-RM) was superior at elevating MPS 24 hours post-RT compared to 30% 1-RM work-matched to the 90% 1-RM leg (240). A 2017 review of light versus heavy loads corroborates these findings and suggests that load may not be as important a variable for increasing hypertrophy, as long as

the set is lifted to voluntary failure (154). The review also notes that lighter loads are typically safer for untrained populations, but moderate loads and rep ranges may be more suitable for focus and time efficiency.

In terms of muscular strength, research has found that training with loads >65% provide a greater stimulus for increasing 1-RM than loads <60% (241). Additionally, the use of heavy loads (90-95% 1-RM) elicited greater strength increases following 8 weeks of training (3x/week) than moderate loads (70-80% 1-RM) (242). However, this study also found that moderate loads were able to increase muscle size greater than heavy loads. A systematic review and meta-analysis compared light versus heavy-load RT (all sets were taken to voluntary muscle failure as part of the analysis inclusion criteria) and the effects on hypertrophy and strength gains (156). The analysis supported previous findings indicating that heavy-load training produced significantly greater strength adaptations, whereas hypertrophy adaptations occurred across a spectrum of loading ranges as long as the sets were taken to voluntary muscle failure (156).

8.1.3 Volume of Exercise

Training volume refers to the total amount of work within a given training session. Volume accounts for total repetitions, sets and load. Higher volume, multi-set (more than 1 set per exercise) protocols are superior in terms of eliciting muscular hypertrophy than single-set protocols (one set per exercise) (152). A meta-analysis by Schoenfeld et al., found that training major muscle groups twice per week yielded greater hypertrophic gains than once per week (151). Another meta-analysis of 55 studies on single versus multiple set training protocols determined multiple sets evoked 40% greater muscular hypertrophy in both trained and untrained subjects (most studies were in young males) than single set programs (152). Moreover, research in untrained males has demonstrated that total volume is more important than frequency of exercise bouts for increasing muscle mass and strength (208). Although the exact mechanism by which multiple-set/higher volume protocols increase positive muscle adaptations is

unknown, it is thought to relate to optimizing total muscle time-under-tension, accumulating greater muscle damage, increasing metabolic stress or a combination of the three (152). A meta-analysis involving 34 treatment groups indicated that a dose-response curve exists for RT volume and muscle hypertrophy (243). The analysis showed that each additional set completed (per muscle group/per week) corresponded to a 0.37% increase in muscle size. The study also indicated that 10+ sets/muscle group/week displayed a greater response in muscle size compared to <5 and 5-9 sets (243).

In regards to strength adaptations, a meta-analysis involving 140 studies revealed that untrained participants can maximize strength gains by training at a minimum 60% of 1-RM, 3 days per week, and 4 sets per muscle group (50). The meta-analysis notes that strength adaptations for novice exercisers occur more rapidly and with less effort (less volume and intensity) than trained individuals as neural adaptations and enhanced motor unit activation typically develop to the greatest extent early on in RT (50).

Increasing volume may also be an effective strategy to increase total energy expenditure within training promoting favourable body composition changes. An acute study found that 3 sets induced greater energy expenditure than only 1 set (244), and that men participating in the multi-set RT session were expending over 200 kcals more during the exercise session compared to the single-set training group (244). Similar research using indirect calorimetry indicated that resting energy expenditure remained elevated above baseline for 72 hours post-RT, which may have implications regarding fat loss (245).

8.1.4 Type of Exercise

Research suggests that multi-joint and single-joint RT exercises both can contribute to muscle hypertrophy and strength gains in untrained men (246). Multi-joint movements are able to invoke greater mechanical tension due to larger loads being moved, as opposed to performing single-joint exercises which are unable to sustain heavy loads (246). However, single-joint exercises allow for

isolation of smaller muscle groups that may not be stimulated entirely during multi-joint movements. Single-joint exercises can specifically target underdeveloped muscles and assist in improving muscular asymmetries (246). Single-joint exercises are more easily learned by untrained subjects, and individual muscle recruitment is enhanced, thus promoting a hypertrophic response (246).

Periodization is another factor that can improve outcomes of a training program. The use of a periodized training program, which introduces variability in the program (i.e. volume, intensity, frequency) has been shown to elicit greater strength and hypertrophic improvements than non-periodized training programs (157–159). The premise behind periodization is that constantly altering the training stimulus will ensure training adaptations continue.

8.1.5 Rest within Exercise

Rest intervals refer to the time spent at rest in between sets. This is another training variable that can be manipulated to maximize the anabolic response of training (247). Short rest intervals of 30 seconds or less generate significant metabolic stress over the course of an exercise session, which can enhance metabolite accumulation and the anabolic response. However, it limits sufficient recovery between sets which reduces performance on subsequent sets (248). Conversely, long rest intervals of 3-5 minutes have been shown to optimize recovery during training and increase performance in subsequent sets (249). However, long rest periods allow for local metabolite removal and a decrease in metabolic stress which can result in reduced muscular hypertrophy (250). Therefore, moderate rest intervals between 1 and 2 minutes may provide an optimal compromise to maintain metabolic stress while allowing for sufficient recovery to maintain performance during subsequent sets (251).

8.2 Screening Questionnaire

Telephone/Verbal FULL Screening Questionnaire

Potential ID number: _____

How did you hear about the study? _____

Please explain the following about the study to the potential participant:

- "This is an **8 week** exercise and nutrition study with a focus on increasing muscle and bone health. We are looking to investigate the effects of two separate supplements (Greek yogurt and a study designed supplement) and exercise (resistance and plyometric training) on muscle size, body composition and bone health in untrained, university-aged males.

- If you are eligible to participate, there is a possibility that you may be asked **to consume Greek yogurt** or a study designed supplement several times a day for the course of the 8 week study period. You will also be asked to participate in a structured exercise program 3 times per week, during the study. The supplement you are asked to consume will depend on what group you are randomized into. Randomization is like flipping a coin – this is how we decide what group you will be in for the rest of the study.

- The study requires a certain time commitment. You will be asked to exercise **3 times per week** with us. The exercise sessions will take place at **Brock University**, and will last about 1 hour, followed by a 1 hour supplement consumption and rest period following exercise (you may bring something to do during this time). The exercise sessions will be created just for you and you will work with a personal trainer. The exercise session will consist of two resistance training (lifting weights) sessions and one plyometric training (jump-based training) sessions. We have all the necessary equipment at Brock to facilitate the exercise portion of the study. At the beginning, middle and end of the study you will record a food diary so we can analyze your diet and nutrient intakes.

- As part of this study, you will be asked to perform several measures at the beginning and end of the study. These measures include having your body composition, weight, height, waist circumference and muscle size analyzed. It also includes 2 tests to assess your strength and power at the beginning and end of the study. Finally, you will be asked to perform 3 blood draws (at beginning, after 1 week and at the end) to analyze markers of bone health. The results of all these tests can be made available to you upon your request. There are also 2 short questionnaires to complete prior to beginning the study.

- We would just like to reiterate that for this study to work to your benefit, we do require a **commitment** from you. It is very important that you **do not miss appointments** for the study and that you try your best to be on time. This is to ensure that we are able to give you the individualized attention and guidance that you deserve from a nutrition and exercise standpoint. We will tell you, the key to success in a study like this is **compliance** and **adherence** to the protocol. We have a lot of experience in running studies like these."

"Do you have any questions?"

"Are you interested in being **formally screened** for the study right now? I would require you to answer a few questions which will take about 5 minutes. Or would you rather think about the information I just gave you and call us back when/if you would like to be screened?"

Interviewer: _____ Date of Contact/Call: _____

Screening Questions:

Potential ID #: _____ Date: _____

D.O.B: _____ Telephone #:_____

e-mail address: _____ Best way to contact you? _____

Interviewer (if different from above): _____

Age:_____ (**must be 18 – 25 years**)

Height: _____ cm

Weight:_____ kg

Calculate BMI (kg/m^2):_____

BMI (**must be Normal**): _____

Underweight	16	18.5
Normal (healthy weight)	18.5	25
Overweight	25	30
Obese	30	+

Exercise frequency:
How many times per week do you perform structured exercise/weight training? _____
(**must be an infrequent exerciser [~ < twice/week] to be eligible**)

Protein supplements consumption:
Do you consume protein supplement products? YES/**NO**

If YES, please specify the types, amount and frequency (servings per day or average per week): _____

Do you consume Calcium and/or Vitamin D supplements and/or multivitamins? YES/**NO**

If YES, amount and frequency: _____
(They would have to go off the supplements for the study. If they do take them, they must have a wash-out period being off them of at least 2 weeks).

As a participant in this study **you may be required** to consume several servings of Greek yogurt or a study designed supplement every day for 8 wks. Are you willing to incorporate either of these into your diet?" **YES**/NO

Does the potential subject meet inclusion criteria? YES/NO.

If YES → It looks like you meet the initial inclusion criteria for the study. Would you mind if I ask you a few more questions about your health history to further confirm your eligibility? This portion will take approximately 5 minutes. (Proceed to Additional Questions for Eligibility)

If NO → Unfortunately, you do not meet the inclusion criteria for the study based on your answer about "X". At this time, we cannot include you in the study*. Thank you for your interest.

***if the reason is because they consume too much supplementary protein (within reason) or a multivitamin/vitamin D/calcium supplement, ask them if they would be willing to not consume these things. If yes, then they can be eligible, BUT they need at least a 2-week washout before we start any pre-measurements.**

Additional Screening Questions:

Date (if different from above): _____

Interviewer (if different from above): _____

Are you a non-smoker? **YES**/NO

Do you consume alcohol on a regular basis (at least every couple of days)? YES/**NO**

Are you lactose intolerant (diagnosed by doctor)? YES/**NO**

Do you believe that you may be lactose-intolerant or lactose-sensitive? YES**/NO**

Do you have an allergy to dairy/milk protein? YES/**NO**

Do you have Celiac Disease? YES/**NO**

Do you have any other gastrointestinal diseases or condition? YES/**NO**

How often do you currently consume Greek yogurt?

Never Once/week Two-three/ week Four-or-more/week

Have you ever had any bone, joint or muscle injury (ACL or knee/hip/lower back injuries, fractures)? YES/**NO**

If YES, when/how did this occur? _____
If YES, how was this treated (i.e. surgery?) _____
If YES, does this currently effect your physical ability (i.e. any limitations)?
_____

Have you ever had any major joint instability or ongoing chronic pain (such as) in the knee, back or elbow? YES/**NO**

If YES, when/how did this occur? _____
If YES, how was this treated (i.e. surgery?) _____
If YES, does this currently effect your physical ability (i.e. any limitations)?
_____

Do you have arthritis or any spinal conditions**?** YES**/NO**

If YES, when/how did this occur? _____

If YES, how was this treated (i.e. surgery?) _____

If YES, does this currently effect your physical ability (i.e. any limitations)?

_____

Other Medical Conditions:

Heart disease/condition	YES/**NO**
Kidney disease/condition	YES/**NO**
Liver disease/condition	YES/**NO**
Pancreatic disease/condition	YES/**NO**
Hepatitis B	YES/**NO**
Hepatitis C	YES/**NO**
HIV/AIDS	YES/**NO**

Do you take any prescription medication? YES/NO

Name and reason: _____

(Anything prescribed by a doctor, i.e. antidepressants or micronutrients are ok)

Do you take any medication that may affect Bone or Muscle? YES/NO

_____

Such as: cortisone, prednisone, Prozac.

Do you take any over-the-counter medications/supplements/vitamins? YES/NO

Name and reason: _____

Do you have any food allergies? YES/NO

Do you fear confined spaces or have claustrophobia? YES/NO

It looks like you have met all of our inclusion criteria. The next step is to schedule an appointment with you to come into the Study Office to discuss the consent form. At this time we can answer any more questions you may have. This appointment should take no more than 30 min. If you would like, we can email you some additional information about the study.

If this potential subject may be ineligible, please consult with a study coordinator before determining final eligibility and before booking an in-person screening visit.

Eligible: YES/NO/consult with coordinator

If NO, give reason: _____

If YES, date of in-person screening/consent visit: _____

Maybe advise all the potential participants to follow washout period (if possible no Greek yogurt consumption and exercise) for two weeks before the intervention.

Additional Comments Below:

8.3 Godin-Shephard Leisure-Time Exercise Questionnaire

ID :_____

GODIN-SHEPHARD LEISURE-TIME EXERCISE QUESTIONNAIRE

1. Considering a **7-day period** (a week), how many times on average do you do the following kinds of exercise for **more than 15 minutes** during your **free-time** (write on each line the appropriate number)?

	Times Per Week
(a) STRENUOUS EXERCISE **(HEART BEATS RAPIDLY)** (i.e. running, jogging, hockey, football, soccer, squash, basketball, cross country skiing, judo, roller skating, vigorous swimming, vigorous long distance bicycling)	_____
(b) MODERATE EXERCISE **(NOT EXHAUSTING)** (i.e. fast walking, baseball, tennis, easy bicycling, volleyball, badminton, easy swimming, alpine skiing, popular and folk dancing)	_____
(c) MILD EXERCISE **(MINIMAL EFFORT)** (i.e. yoga, archery, fishing from river bank, bowling, horseshoes, golf, snow-mobiling, easy walking)	_____

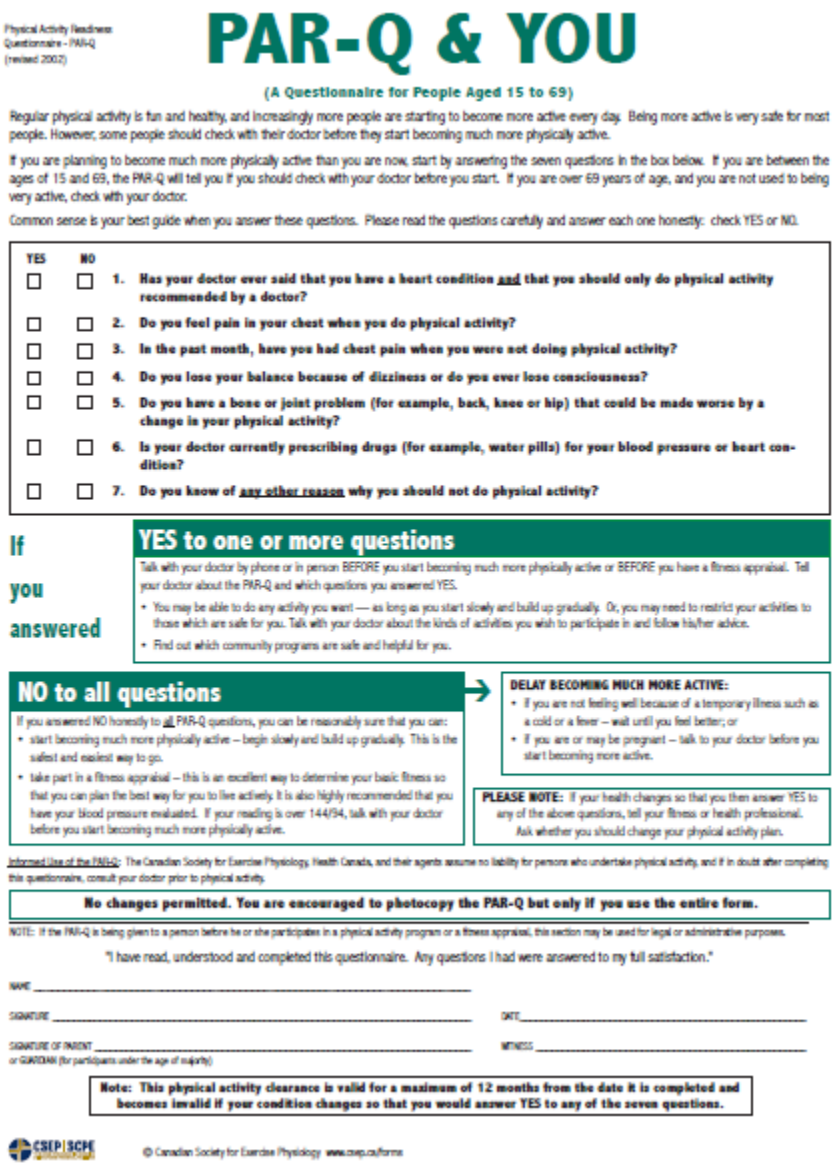
2. Considering a **7-day period** (a week), during your leisure-time, how often do you engage in any regular activity long enough to work up a sweat (heart beats rapidly)?

1. OFTEN	**2. SOMETIMES**	**3. NEVER/RARELY**
☐	☐	☐

3. Considering a **7-day period** (a week), during your leisure-time, how often do you engage in any weight-lifting or other types of training with the goal to build muscle or increase physical performance (1.e. plyometric training) activity long enough to work up a sweat (heart beats rapidly)?

1. OFTEN	**2. SOMETIMES**	**3. NEVER/RARELY**
☐	☐	☐

8.4 Physical Activity Readiness Questionnaire



Physical Activity Readiness Questionnaire - PAR-Q (revised 2002)

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO	
☐	☐	**1. Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?**
☐	☐	**2. Do you feel pain in your chest when you do physical activity?**
☐	☐	**3. In the past month, have you had chest pain when you were not doing physical activity?**
☐	☐	**4. Do you lose your balance because of dizziness or do you ever lose consciousness?**
☐	☐	**5. Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?**
☐	☐	**6. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?**
☐	☐	**7. Do you know of any other reason why you should not do physical activity?**

If you answered

YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active – begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal – this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

DELAY BECOMING MUCH MORE ACTIVE:

- If you are not feeling well because of a temporary illness such as a cold or a fever – wait until you feel better; or
- If you are or may be pregnant – talk to your doctor before you start becoming more active.

PLEASE NOTE: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

Informed Use of the PAR-Q: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME _____

SIGNATURE _____ DATE _____

SIGNATURE OF PARENT or GUARDIAN (for participants under the age of majority) _____ WITNESS _____

Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.

CSEP | SCPE

8.5 Food Diary

For Office Use Only

Initials Entered ____

Initials Proof ____

B.E.A.S.T. (Brock Exercise and Supplement Trials):
Effects of Two Different Supplements and Exercise on Muscle and Bone Health

Please return to WH144 office by:

_____

RECORD OF FOOD INTAKE

ID: _____ Date: _____ (week ____)

Day of Week (circle): Mon Tue Wed Thu Fri Sat Sun

MEAL/ TIME	**FOOD CONSUMED**	**AMOUNT** – e.g. cups, mL, grams, ~~oz~~, ~~tbsp~~, tsp, servings, portion	**HOW IT WAS PREPARED** - broiled, baked, steamed, fried with olive oil, …	**SEASONINGS/ TOPPINGS**	**NOTES** Brands, recipes of homemade food, or any other detail not described

Actual food diary was full page and consisted of 3 or 7 identical pages corresponding to number of days the food diary.

8.6 Brock University Research Ethics Board Clearance

Brock University
Research Ethics Office
Tel: 905-688-5550 ext. 3035
Email: reb@brocku.ca

Bioscience Research Ethics Board

Certificate of Ethics Clearance for Human Participant Research

DATE:	7/4/2017		
PRINCIPAL INVESTIGATOR:	JOSSE, Andrea - Kinesiology		
CO-INVESTIGATOR(S):	Brian Roy (broy@brocku.ca); Wendy Ward (wward@brocku.ca)		
FILE:	16-295 - JOSSE		
TYPE:	Masters Thesis/Project	STUDENT:	Aaron Bridge
		SUPERVISOR:	Andrea Josse

TITLE: Effects of Greek yogurt and Exercise on muscle size, body composition and bone health in untrained, university-aged males

ETHICS CLEARANCE GRANTED

Type of Clearance: NEW Expiry Date: 7/1/2018

The Brock University Bioscience Research Ethics Board has reviewed the above named research proposal and considers the procedures, as described by the applicant, to conform to the University's ethical standards and the Tri-Council Policy Statement. Clearance granted from **7/4/2017** to **7/1/2018**.

The Tri-Council Policy Statement requires that ongoing research be monitored by, at a minimum, an annual report. Should your project extend beyond the expiry date, you are required to submit a Renewal form before 7/1/2018. Continued clearance is contingent on timely submission of reports.

To comply with the Tri-Council Policy Statement, you must also submit a final report upon completion of your project. All report forms can be found on the Research Ethics web page at http://www.brocku.ca/research/policies-and-forms/research-forms.

In addition, throughout your research, you must report promptly to the REB:

a) Changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study;
b) All adverse and/or unanticipated experiences or events that may have real or potential unfavourable implications for participants;
c) New information that may adversely affect the safety of the participants or the conduct of the study;
d) Any changes in your source of funding or new funding to a previously unfunded project.

We wish you success with your research.

Approved: Sandra Peters

Sandra Peters, Chair
Bioscience Research Ethics Board

Note: Brock University is accountable for the research carried out in its own jurisdiction or under its auspices and may refuse certain research even though the REB has found it ethically acceptable.

If research participants are in the care of a health facility, at a school, or other institution or community organization, it is the responsibility of the Principal Investigator to ensure that the ethical guidelines and clearance of those facilities or institutions are obtained and filed with the REB prior to the initiation of research at that site.